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ON DICHLOROETHYLSULFIDE (MUSTARD GAS)

IV. THE MECHANISM OF ABSORPTION BY THE SKIN

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1. INTRODUCTION

The purpose of this paper is to present experimental data bearing on the absorption of dichloroethylsulfide by the skin, and to discuss in a preliminary form the mechanism of penetration.

The chemical and physical properties of the pure compound are briefly as follows: It is a colorless oil, boiling at 217°C. (760 mm.) M. P., 14.5°; V. P., 0.06 mm. Hg. at 20°C.; density, 1.274 at 20°C. In the crude state it possesses a faintly garlic-like odor, and impurities color it a straw color to a distinct pink or brown. It is immiscible with and only very slightly soluble in water (0.07 per cent at 10°C.,) but it is miscible in all proportions with alcohol and other organic solvents such as ether, benzene, xylene, turpentine, chloroform, etc. In the pure state it is neutral to litmus. A saturated water solution has been shown to hydrolyze rapidly yielding hydrochloric acid and probably dihydroxyethylsulfide.

2. CHANNELS OF ABSORPTION

At an early stage in these investigations it was observed that after exposure of the skin to either the liquid or vapor the most efficient method of removal was washing with some solvent of low volatility, such as kerosene, followed by thorough rinsing with soap and water. With such a treatment, it was found possible to apply the oil to the skin, and by washing after two or three minutes, to either prevent the subsequent delayed reaction entirely, or to reduce it to a slight hyperemia. The very narrow time limits within which these preventive measures were effective indicated a remarkably rapid absorption of at least a part of the *gas*. It was found, however, that if the sponging with kerosene was continued vigorously for twenty to thirty minutes, with frequent renewals of both sponge and solvent, vesication could be prevented in most cases after an exposure of 10 minutes or less and in rare instances after 15 minutes (table 1).

The following tests were made on the shaved and dried skin of the forearm by the "Nail-head Method." A nail-head 3 mm. in diameter was pressed firmly against filter paper saturated with dichloroethylsulfide and then applied with firm even pressure to the skin. After a definite period of time the exposed area was washed or "treated." This period of undisturbed contact between the oil and the skin (or, in subsequent experiments, between the vapors and the skin) will hereafter be referred to as the length of exposure. Since the reaction does not appear for several hours, and since the burns do not develop sufficiently to permit of accurate comparison in less than twenty-four hours, the first readings were made at the expiration of that period, and final readings after forty-eight hours. The reactions as observed at that time are graded as follows:

- + Mild erythema
- ++ Moderate erythema and swelling.
- +++ Mild vesication with slight surrounding erythema.
- ++++ Severe vesication with slight surrounding erythema.
- +++++ Severe vesication with marked surrounding erythema and edema.

TABLE 1

Removal of dichloroethylsulfide from skin by washing with kerosene

SUBJECT	LENGTH OF EXPOSURE	KEROSENE TREATMENT	DEGREE OF BURN AFTER 24 HOURS
	<i>min.</i>	<i>min.</i>	
B. E.....	10	10	++++
A. H.....	10	10	++++
E. W.....	10	10	+++++
P. E.....	10	15	++++
H. A.....	10	15	+++
E. C.....	10	15	+++
M. C.....	10	20	+
C. R.....	10	20	++
E. C.....	10	20	+
B. R.....	20	30	++++
G. R.....	20	30	++++
B. C.....	30	30	++++
K. E.....	90	60	+++++
L. Y.....	10	10	+++
L. Y.....	10	30	+
H. E.....	10	10	+++
H. E.....	10	45	+

It should be noted in the table that +++ indicates the penetration of sufficient mustard to cause a blistering burn while + and ++ indicate that the amount of mustard introduced is insufficient to cause a blister, but only redness and slight swelling. Beyond the limits given above it is unsafe to make quantitative comparisons of two or more burns.

A ten minute exposure, if washed with kerosene for 10 minutes or less, blistered; the actual blistering could be prevented in most cases by washing for 15 minutes or more. Little added benefit could be obtained by treating longer than 25 to 30 minutes. Where the time of exposure was increased to 15 minutes or more vesication could rarely be prevented, even after rubbing or sponging for an hour.

That dichloroethylsulfide was actually being removed from the skin after the first few minutes of treatment was shown by the observation that unless the sponge and kerosene used were frequently changed, and the surrounding areas thoroughly washed the resulting burn was greatly increased beyond the size of the original exposure—following in general the areas wet by the kerosene—and in some cases even the operator's fingers became contaminated with the *gas* and a burn resulted.

Prolonged washing was tried with soap and water after ten to fifteen minutes in a similar manner, but though some benefit was observed, it was not nearly as marked as when the washing was done with kerosene or some solvent for the oil. (Vaseline, acetone, alcohol and benzene were tried.)

From these observations, it is quite evident that the dichloroethylsulfide is at first rapidly taken up by some element on—or adjacent to—the surface of the skin, and for 2 to 3 minutes it may be completely removed, and for 10 to 15 minutes partially removed by prolonged washing with an organic solvent, and to a lesser extent, with soap and water. This conclusion has been emphasized because it is an important consideration in dealing with the ultimate absorption of the substance.

In the absence of accurate information regarding the physical and chemical constitution of the outer layers of the epidermis and the exact location and condition of the *mustard gas* when first taken up, it appears inadvisable to speculate as to whether the *gas* is at first adsorbed on the skin surface and then penetrates the sweat glands by capillarity or absorption, or a mixture of both, or whether it passes to a certain extent directly through the cortical layers by a process of absorption or solid solution. Substances like keratin might be expected to adsorb the compound and fats and lipoids to absorb or dissolve it. It is sufficient for the moment to consider the skin as a protective medium taking up the compound on its surface and offering a definite resistance to passage of the *gas* inward. This resistance may be gauged by determining the amount of *gas* which it is necessary to apply externally to insure the delivery to the inner tissues of a concentration sufficient to produce recognizable toxic effects. The nature of this resistance will become evident after the discussion of the loss of the *gas* from the skin by evaporation.

3. RELATION OF TIME OF EXPOSURE TO CONCENTRATION

It is now a well established fact that individuals vary in their sensitivity to slight quantities of dichloroethylsulfide (1). This variation between individuals can be easily demonstrated by determining the shortest exposure to the vapors which will produce a visible reaction.¹ Since any individual gives an increasingly severe reaction with increasing length of exposures until the full effects of the irritant are obtained (severe vesication), there should be a definite relationship between the concentration of the dichloroethylsulfide, and the length of the exposure required to produce burns of equal intensity. The method used in working out this relationship² was based upon that used in "gassing chambers" (2).

The principle is briefly as follows: Dry air is bubbled through dichloroethylsulfide at a known and constant rate, and mixed with a definite quantity of dry air also under constant flow for the purpose of dilution (fig. 1). A suitable glass exposing chamber is placed in the circuit near the bubbling-tube, using as little rubber tubing as possible for connections. (This substance absorbs the gas very readily.) The concentration may be determined by analysis of the escaping gas and air mixture from the open end of the exposing chamber, but can be accurately and more readily estimated by the loss-in-weight method, figuring the loss in weight of the bubbling tube after a certain period of time in relation to the known quantities of air employed. The skin of the forearm of the subject is shaved and sponged with alcohol and the desired exposures are made. Stress

¹ The details for determining sensitivity have been described in the communication referred to above (1). The "test-tube method" consists of saturating a cotton plug, mid-way of a small test-tube, with liquid dichloroethylsulfide. This smaller tube is immersed in a larger tube filled with water to maintain a constant temperature. While some men will give a distinct reaction to a 10 to 15 second exposure at (20°) with such a tube, others require 5 to 10 minutes. Such types are subsequently referred to as *sensitive* and *resistant*.

² Devised by Lynch, V., Williams, J. W., and Barba, P. S., May 1918, American University Experiment Station. The first form of application chamber used was a stop-cock with the plunger removed. The form illustrated was devised by Dr. P. J. Hanzlik.

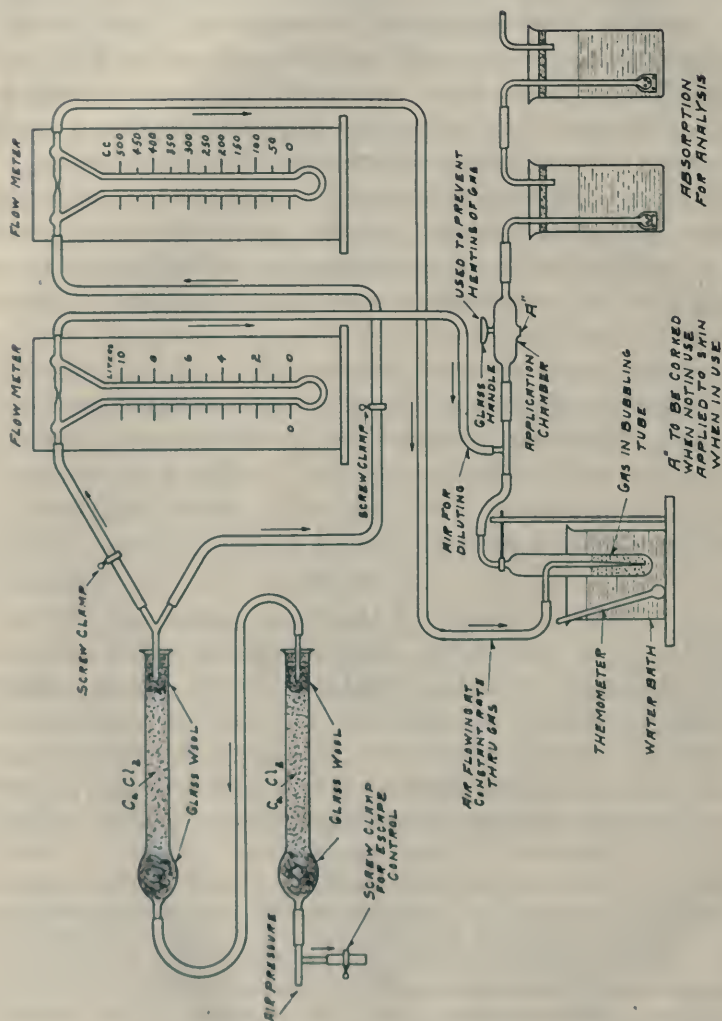


Fig 1

SKIN IRRITANT VAPOR APPARATUS
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is again laid on the fact that readings are made after twenty-four and again after forty-eight hours, since the burns do not develop fully until that time. A faint disk of erythema is considered positive.

Two curves are given below (fig. 2 and fig. 3).

While these curves are both apparently hyperbolic in form and consequently asymptotic, there appears to be a critical level in concentration at approximately 0.002 to 0.005 mgm. of gas per liter at which no injurious effects are noted after prolonged exposures. From this we must conclude that the amount of gas finding its way into the interior tissues after an exposure to the concentrations in question is no more than can be metabolized by the cells. This figure corresponds with previous observations regarding the threshold concentration of gas that can be disposed of without injury in prolonged respiratory tests.

We consider it essential to mention here that in determining the toxicity of dichloroethylsulfide on animals by inhalation a similar phenomenon is observed; data expressing the relation of time to concentration indicate a marked critical condition at this same mean concentration, 0.005 mgm. per liter. While the data for different species indicate differences in sensitivity, they all indicate an ultimate threshold concentration within the limits of 0.01 and 0.001 mgm per liter. It seems evident that this concentration represents a border line where the organism generally can metabolize the poison without serious effects.

It must be remembered that these experiments were originally carried out with a purely practical military objective: The determination of the relation between the concentration of gas and the time of exposure required to produce a burn under field conditions. We were well aware that the loss from the skin by evaporation was considerable, and that exact scientific data regarding absorption of the gas by the skin and its penetration into the interior tissues could only be secured by covering the exposed area with some impervious substance immediately after the completion of treatment. Unfortunately, the suspension of experimental work on cessation of hostilities has prevented our carrying out an elaborate series of experiments on these lines with

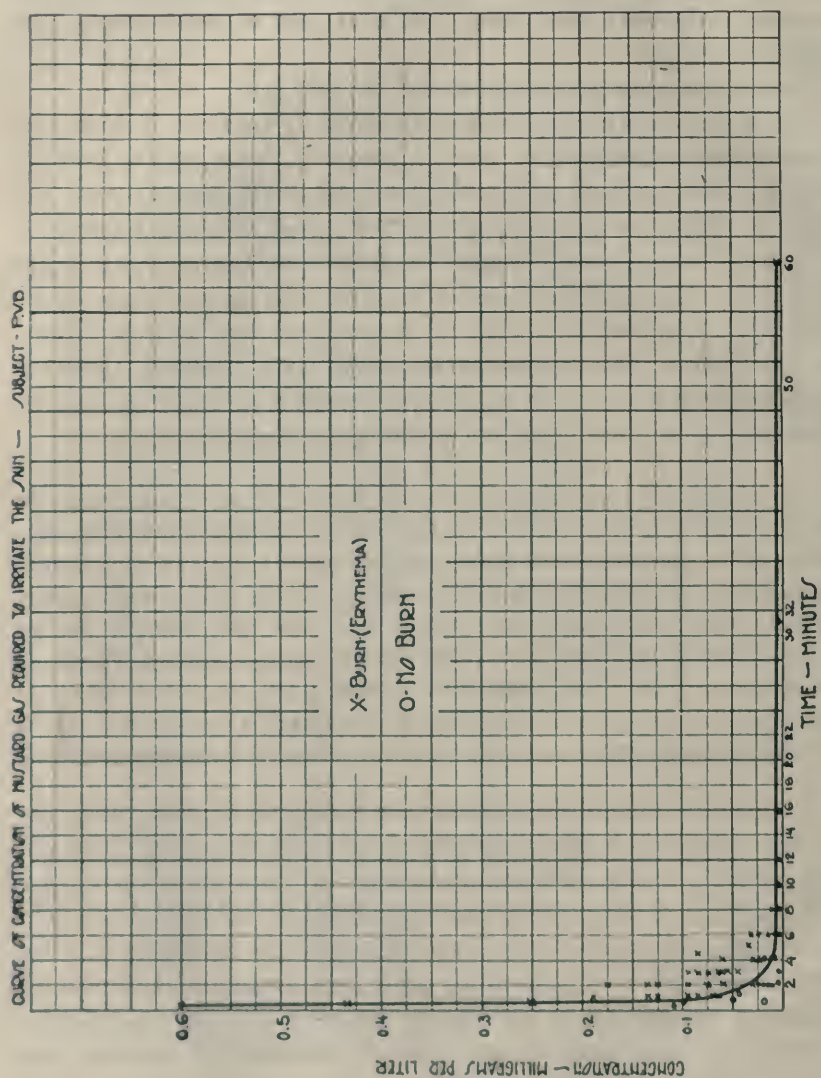


FIG. 2. RELATION OF TIME AND CONCENTRATION IN THRESHOLD BURNS ON P. V. B.

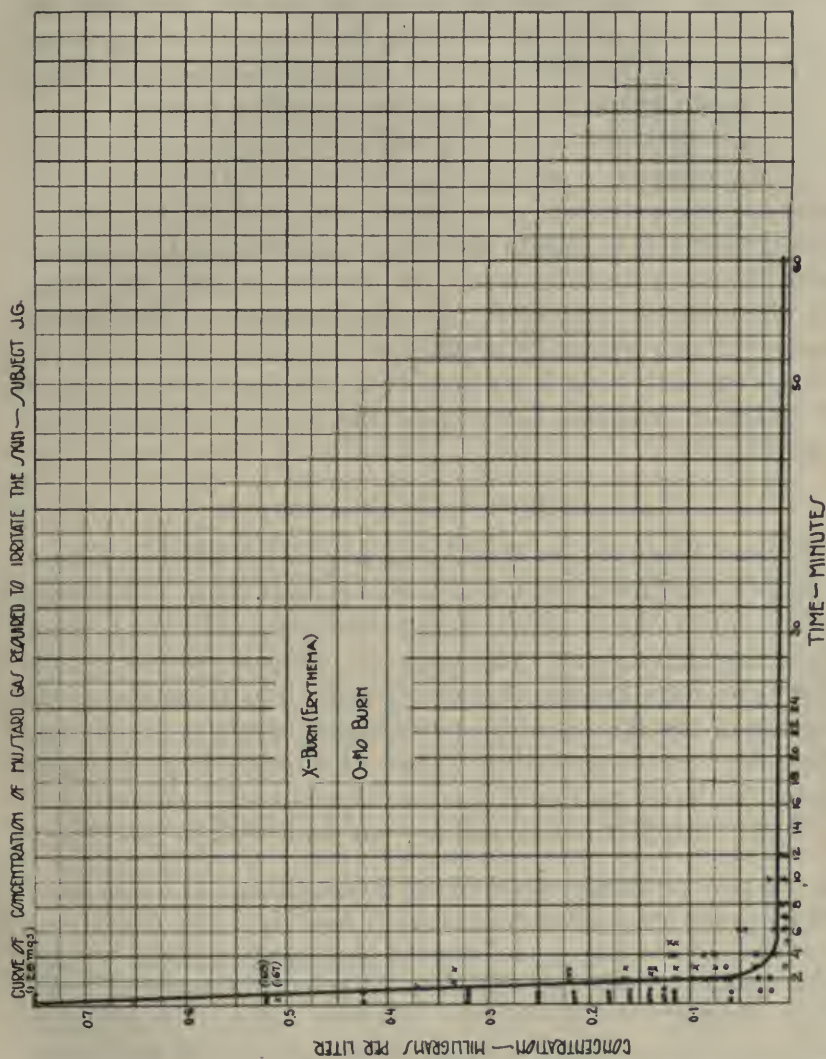


FIG. 3. RELATION OF TIME AND CONCENTRATION IN THRESHOLD BURNS ON J. G.

varying concentrations of the compound. But from experiments with one concentration, to be reported in a subsequent section of this paper, it has been demonstrated that the loss from the skin is extremely great, far exceeding the amount that penetrates the tissues, and for this reason it appears inadvisable to draw any far reaching conclusions from the preceding curves.

The fact that a definite proportionality appears to exist between the increase in time at a given concentration and the increase in concentration at a given time required to produce equivalent effects in several individuals suggests that the problem is one of satisfying the capacity of the skin to such an extent that the requisite amount of gas may penetrate to the interior tissues. The question as to whether this difference is due to a larger capacity or a lower affinity on the part of the skin of the resistant man has been made the subject of further experiments.

4. EFFECTS OF EVAPORATION

We must be careful in considering any factor which is known to modify the final intensity of a *mustard gas* burn, to differentiate between those which *modify absorption only* and those which may have some *effect upon the ultimate reaction* which follows the introduction of the substance into those organized elements of the skin in which pathological effects are produced. Where the former may be brought about through simple physical means, the latter no doubt involve physiological relations of a much more complex nature and are not dealt with in this paper.

As we have previously explained, we do not attempt to specify what agencies are involved in the preliminary or final absorption of dichloroethylsulfide by the skin. The detailed localization of these agencies we have not found necessary to a clear conception of the general mechanism. Passing from the external absorbing agent which we have previously indicated, the dichloroethylsulfide would in all probability pass through the deeper lying elements to the lipoid constituents of the cells of the adjacent tissues. If we are dealing with true *absorption* into the intermediary binder, the passage to the deeper tissues would probably be by diffusion from solid solution; on the other hand, its

retention in this intermediary binder by *adsorption* would make it highly probable that capillarity and surface tension effects would direct it into the duets, from which it would be taken up by absorption. On reaching the lipoids of the cell wall or cell contents proper, the *mustard gas* would slowly diffuse into the aqueous constituents of the cell, where hydrolysis of the compound would result and its toxic action would begin. Again we must call attention to the danger of attempting to localize the site or mechanism of this action from the evidence on hand. The nature of the poison may be such as to effect a fatal anaesthesia of the vaso-motor nerve supply, resulting in prolonged vaso-dilation and subsequent edema and vesication. On the other hand there is abundant evidence from the work done by Lillie, Clowes and Chambers on the penetration of marine eggs to indicate that the intracellular liberation of acid which would follow the hydrolysis of the dichloroethylsulfide would cause such profound changes in the protoplasmic equilibria of the cells concerned that their normal metabolism would be permanently upset and their permeability increased (3). We are forced to believe, from the evidence on hand, that equal concentrations of the poison in this last phase (inside the cells of the tissues affected) would produce similar and nearly equal reactions in almost all men. Whereas the extreme difference in sensitivities of the skin represents an order of 1 to 600, the extreme difference in toxicity for any one species, (dog) by inhalation or injection, is of a much smaller order, possibly 1 to 5. Exceptions to this equality of response to equal intracellular concentrations have been noted. One such case, where the subject (H. W. S.) has developed an abnormal reaction suggesting an anaphylactic condition is described fully in a previous paper (1).

It has been suggested that after exposure of the skin to small amounts of *mustard gas*, evaporation from the surface has an important bearing upon subsequent absorption. To test this point exposures to the vapors were made with the test-tube method previously described. Two exposures, just sufficient to cause a mild reaction were made at the same time and under identical conditions. One was immediately covered with a shallow glass thimble of approximately the same diameter as the exposed area, fastened to the arm for three hours or more with adhesive tape. The other was left uncovered, and served as a control. After twenty-four to thirty-six hours when both

burns had reached a maximal comparative development, the covered burn was seen to be much the worse. A series of experiments performed in this manner is tabulated in table 2.

Column I—Subjects average minimum burning time to the Standard Test-tube, 20°C.

Column II—Length of exposures made.

Column III to IV—Relative intensity of resulting burns, + + + severe erythema; + + mild; + faint; — negative. (Blistering burns were not made in any case).

TABLE 2

SUBJECT	I SENSITIVITY	II EXPOSURE	III COVERED	IV UNCOVERED
C. R. M.....	3 to 4 min.	4 min.	+++	++
F. W. W.....	3 to 4 min.	3 min.	+++	+
E. G. S.....	2 to 3 min.	3 min.	+++	+
J. W. W.....	30 sec.	20 sec.	+	—
H. W. S.....	10 sec.	10 sec.	+	+

Sensitive men (i.e., men who give a positive reaction to short exposures) generally show less increase in reaction than do men who are relatively much more resistant. This suggests that the minimal burning time be determined when the exposed skin is subsequently covered for a prolonged period and compared with the minimal burning time when exposures are left uncovered.

From table 3 it is apparent that the possible reduction in minimal burning time in a sensitive individual by covering the exposure is generally less than in a resistant individual.

This suggests that the skin of a resistant individual absorbs more of the dichloroethylsulfide than the skin of a sensitive one. Because, the only other apparent way in which covering could influence the final reaction would be by increasing the rate of passage of the sulfide from the outer layers of the skin to the inner layers through an increase in temperature and thus raise what would naturally (uncovered) be a subreactive concentration to a reactive one. On such a basis it would seem obvious that a sensitive man would show relatively as great an increase in intensity as a resistant man. Against the argument that a sen-

sitive man exhibits a close approach to the maximum reaction, and therefore cannot be made to show a large increment over his normal reaction, (uncovered), is the fact that individuals are known who *blister* to a *five second vapor exposure*, and give severe *edema* to less than *one second*, and in whose history there is nothing to indicate any anaphylactic or other abnormal reaction, beyond their great sensitivity. This problem will be considered later.

TABLE 3

SUBJECT	WHEN LEFT UNCOVERED	COVERED IMMEDIATELY FOR 10 HOURS	
	Minimal burning time	Length of exposure	Degree of burn
		<i>seconds</i>	
G. G. F.....	3 minutes	1	Negative
		5	Negative
		15	Positive
		30	Positive
		60	Positive
C. R. M.....	4 minutes	30	Positive
F. W. W.....	4 minutes	15	Positive
		30	Positive
E. G. S.....	2 to 3 minutes	30	Positive
P. S. B.....	20 seconds	1	Positive
		5	Positive
		10	Positive
H. W. S.....	10 seconds	2	Negative
G. W. W.....	30 seconds	2	Negative
		20	Positive

Another point of interest is the time an exposure must subsequently be covered to secure the maximum reaction on any one individual, thus finding the time during which the loss by evaporation will still produce an appreciable decrease in the final intensity of the burn. An experiment to determine this is given in table 4. Exposures of border-line intensity were made and covered immediately, and were left covered for varying intervals.

In another experiment (table 5) the approximate rates of evaporation and absorption were determined by covering mild exposures after varying intervals of time had elapsed during which the skin had been left open to the air.

From these experiments it is evident that equilibrium in the skin is reached in about 45 minutes and that capping has less effect on a sensitive than on a resistant skin. This rate of attaining equilibrium must have a direct bearing upon further absorption and, ultimately, upon sensitivity. Since the dichloroethylsulfide is at first absorbed by the surface of the skin or some superficial elements adjacent thereto, and can be lost from this medium by evaporation, the relative rate at which it passes from this intermediary binder to some other phase, in which it is firmly fixed, as against the rate at which it will be lost by evaporation; will

TABLE 4

Burns were covered immediately and left covered for varying intervals as indicated

SUBJECT	SENSITIVITY	EXPOSURE	TIME COVERED			
			15 min.	30 min.	45 min.	60 min.
C. R. M.....	4 min.	5 min.	+	++	+++	+++
H. W. S.....	10 sec.	5 min.	++	++	++	

TABLE 5

Burns were covered after varying intervals had elapsed and left covered for six hours

SUBJECT	SENSITIVITY	EXPOSURE	LEFT UNCOVERED	COVERED AFTER EXPOSURE TO AIR FOR				
				0 min.	15 min.	30 min.	45 min.	60 min.
H. E. I....	3 min.	3 min.	+	++++	+++	++	+	+
H. W. S...	20 sec.	15 sec.	-	+++	+	+	-	-

be the determining factor in the question of whether or not a slight exposure to the vapors will prove positive or negative.

Since the intensity of the burn produced by a given concentration bears a direct relation to the length of the exposure, and since in at least one case, (H.W.S.) a reaction has been produced within ten minutes of exposure, it is probable that the velocity with which the substance passes from the surface to the deeper layers is such that the absorption of additional amounts is not interfered with. While the determination of the rate at which it passes from the surface inward is a problem of considerable complexity, the rate at which it is lost from the skin by evap-

oration can be readily determined by a few simple experiments. If we make a series of exposures of varying periods of time, and cover certain of these exposures to prevent evaporation, it will be possible to determine the time for which a short exposure must be covered to give a burn of equal intensity to an exposure of greater length which has been left open to the air.

Two such experiments were made on the skin of the shoulders of two subjects, (H.E.I. and W. B.Mc.); the burns were covered immediately and left covered for the time given in the left-hand columns, tables 6 and 7. We have endeavored to give an accu-

TABLE 6

Effect of covering exposures of varying lengths. H. E. I. Minimum burning time, 1 minute

TIME COVERED	LENGTH OF EXPOSURE				
	15 seconds	30 seconds	1 minute	2 minutes	3 minutes
0.....	—	—?	+	++	+++
15 seconds.....	—	—?	+	++	
30 seconds.....	—	++?	++	+++	
1 minute.....	—	++	+++	+++	
*2 minutes.....	++?	+++?	+++?	++	
*4 minutes.....	+	+++?	+++?	++	
16 minutes.....	+++?	+++	++++	++++	
32 minutes.....	++	+++	++++	++++	
64 minutes.....	+++	++++	++++		
128 minutes.....	+++				

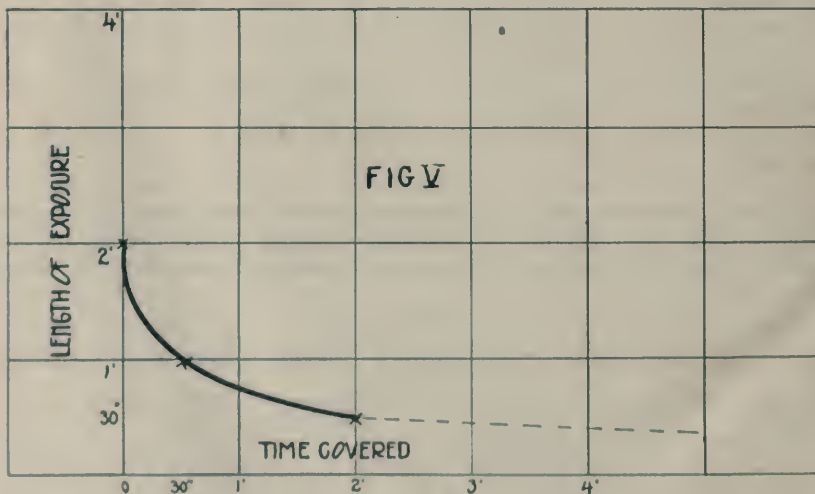
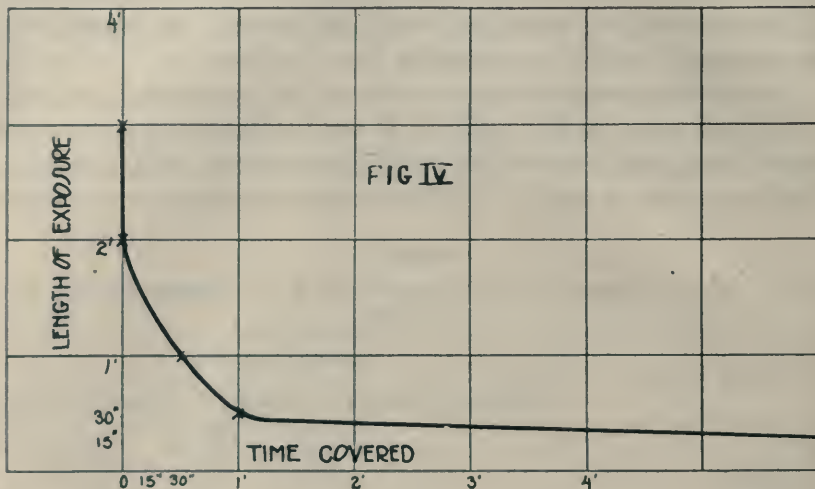
* In the exposures capped for 2 and 4 minutes it is probable that the thimbles were not tight. The skin used was on a curved surface of the shoulder, and the resulting burns are undoubtedly low in intensity through this experimental error.

TABLE 7

Effect of covering exposures of varying lengths. W. B. Mc. Minimum burning time, 1 minute

TIME COVERED	LENGTH OF EXPOSURE				
	15 seconds	30 seconds	1 minute	2 minutes	4 minutes
0.....	—	—?	+	++	+++
30 seconds....	—	++?	++	++	
2 minutes.....	—	++?	++		
8 minutes.....	++?	++			

rate representation of their relative severity after they had reached their maximum comparative development, as determined by the readings of three observers. The system of nom-



enclature is simply an effort to give the relative severity of the burns in each set, and comparisons cannot be made from one set to the other.

Where table 6 is a comparison of more intense burns, table 7 is a comparison of threshold burns, i.e. where the exposure was just sufficient to produce a faint reaction. Figures 4 and 5 give these results plotted as curves.

Since a 15 second exposure can be increased by covering to the intensity of a 4 minute exposure, the amount lost by evaporation into the air must be far in excess of that which passes into the deeper tissues. Our experimental evidence is at present insufficient to determine the relative distribution between air and the skin but we hope to secure further information on this question in the near future. The practical importance of the fact of evaporation cannot be overlooked, and its bearing on variations in cutaneous sensitivity will now be considered from another point of view.

5. TRANSFER FROM SKIN TO SKIN

An interesting phenomenon is observed when the untreated normal skin of one subject is impressed for 5 minutes upon an area of skin of another subject which has been exposed previously to the vapors of dichloroethylsulfide. Under these circumstances, both *donor* and *recipient* may develop burns (due to the transposition of the poison from one skin to the other) the intensity of which will vary according to the circumstances and the respective sensitivities of the participants. The degree of transposition is most strikingly observed in the intensity of the *burn on the donor's arm*. If two similar exposures are made on the arm of a sensitive man, and one of these burns is treated, so to speak, by contact for five minutes with the skin of a resistant man, the treated burn will be markedly less severe than the control, in some cases being entirely prevented. If, however, the recipient is equally sensitive to or more sensitive than the donor, the burns on the latter will exhibit far less difference. Both treatments may be effected at once, using two recipients, one more resistant, and one less resistant, than the donor. In such a case *the burn brought in contact with the more resistant skin will be the less severe*.

A few experiments of this nature are given in table 8. The subjects are divided for clarity into two classes, (R) resistant and (S) sensitive. It is evident that the "resistant skin" has reduced the burn with which it was in contact much more than has the "sensitive skin." We are forced to the conclusion that it has actually *absorbed more of the gas*.

TABLE 8

DONOR	SENSITIVITY	RECIPIENTS	SENSITIVITY	BURNS EXHIBITED BY	
				Donor	Recipient*
H. F. S.....	(S)	Treated by J. A.	(S)	++++	++
		Treated by C. R. M.	(R)	+	+
H. E. S.....	(R)	Treated by J. A.	(S)	++	++
		Treated by C. R. M.	(R)	+	+
J. W. W....	(S)	Treated by H. W. S.	(S)	++	
		Treated by C. R. M.	(R)	+	

* It was found that if the burns on the recipients were capped, they would develop to approximately the same intensity, showing that the recipient's sensitivity conditions the final intensity of his burns, as might be expected.

This same experiment can be carried out in the reverse order. Similar exposures are made on the arms of a sensitive and a resistant subject. Immediately after the exposure, a third subject *takes* both burns by impressing them against his arm for five minutes. It is seen that a "sensitive skin" will *take* more of the poison from a "skin of equal sensitivity" than from a more resistant one.

TABLE 9

DONOR	SENSITIVITY	RECIPIENT	SENSITIVITY	INTENSITY OF THE BURNS ON RECIPIENT
1. C. R. M.....	(R)	H. W. S.	(S)	1. +
2. J. W. W.....	(S)			2. +++
1. C. R. M.....	(R)	E. K. M.	(S)	1. +
2. J. W. W.....	(S)			2. ++++

Both experiments are, in fact, important evidence that the skin of a resistant individual exhibits a greater affinity or capacity for dichloroethylsulfide than that of a sensitive one. Whatever the arrangement of the experiment, the results indicate that there is an actual partition of the *gas* between the two skins, with an evident tendency to establish an equilibrium in which the largest portion of the *gas* will remain in that skin which possesses the greater capacity for it. The experiment confirms in a striking manner the observations noted above (pages 8 and 14), and explains why equilibrium with the air is attained from a sensitive skin sooner than from a resistant one. This is directly contrary to the general belief that a resistant individual is more resistant to low concentrations of *mustard gas* than his fellows because his skin absorbs less. The assumption that his skin absorbs more makes it imperative that some explanation accounting for the paradox be made.

In order to determine the persistence of the *gas* on the surface of the skin after exposure, an experiment was made in which a series of 5-minute exposures were capped for 15 minutes, 30 minutes, 45 minutes, etc., and then impressed on the arm of a subject of about the same sensitivity for 5 minutes.

TABLE 10

	TIME CAPPED			
	15 minutes	30 minutes	45 minutes	60 minutes
Donor C. R. M. (R).....	+	++	+++	+++
Recipient R. S. (R).....	++	++	+	-

Since the recipient fails to develop a burn after contact with the burn on the donor which had been capped for 60 minutes, it is apparent the gas has disappeared from the donor's arm in that period. This agrees well with the results obtained by capping in table 5, in which a maximum absorption is indicated within that time.

6. RELATION OF PHYSICAL PROPERTIES TO PENETRATION

It is generally recognized that substances possessed of the capacity of readily penetrating protoplasm are almost invariably soluble to a certain extent in water and are also "lipoid soluble"—that is, soluble in fats and organic solvents such as benzene and xylene, and that the so-called partition coefficient between water and benzene or xylene is a factor of considerable importance in conditioning their power to penetrate living cells.

We have investigated some twenty-five compounds, so-called *war-gases* which are not only highly toxic, but which irritate the skin. The determination of their partition coefficients is a very difficult matter in most cases because of their rapid hydrolysis in water, (in such cases a strong acid is one of the decomposition products) but it is evident that they are all soluble in both lipoid solvents and water to some extent. This fact, with the fact of their hydrolysis, suggests that the mechanism of their action may be correlated in a general way with that of dichloroethyl-sulfide. Further experimental work is necessary to establish this fact. This group of compounds in the category of *war-gases* vary greatly in their degree of activity and in their specific toxic effects, and while no far reaching generalization on this question will be attempted it is proposed at a subsequent stage in this paper to discuss briefly certain cases in which toxic effects correlate in a measure with physico-chemical properties.

A series of experiments was performed with a number of organic bases in an attempt to determine their value as a means of counteracting the effects of *mustard gas*. It was found that with the exception of *ammonia*, only those bases which were lipoid-soluble irritated the skin, indicating penetration. On the other hand, too high a partition coefficient (too low solubility in water) appeared to diminish the irritating activity on the skin. (The following examples are representative of the groups tried: *ammonia*, *propylamine*, *ethylamine*, *amylamine*, *di-isoamylamine*, *bornylamine*). Whatever the interpretation of the details, it is apparent that lipoid solubility is an important factor in the penetration of the intact skin.

A brief reference should be made to a phenomenon requiring further investigation. Field observers have noted that burns occur more frequently on moist than on dry portions of the body. This observation has been confirmed in the laboratory and furthermore a film of water on the dry skin has been found to facilitate the passage of *mustard gas* showing that the effects observed in the field are not attributable simply to a possible higher permeability of freely perspiring areas but is in some way attributable to the presence of water on the skin.

To determine whether this was attributable to surface effects caused by the presence of a film of fluid a series of equal threshold burns were made over the dry skin, and skin wet with various organic agents, care being taken in the case of each individual to select the exact time of exposure required to give a mild burn on the dry skin.

TABLE 11

PRELIMINARY SKIN TREATMENT	DEGREE OF BURN						
Dry.....	+	+	+	+	+	+	+
Washed soap and dry.....	+						
Film of H ₂ O.....	++	++	++	++	++	+++	++
Film of Alcohol.....	++	++	++	++			
Film of Ether.....	++		++	++			
Film of Xylene.....	++	++	++	++			
Film of Kerosene.....	++?		++	++?			
Subject.....	G. C.	A. C.	A. H.	H. S.	V. L.	H. S.	E. M.

The fact that water is a very poor solvent for *mustard gas* and yet appears to facilitate the passage of the substance into the tissues as well as the other fluids which are good solvents for it suggests the probability that capillarity rather than solution may play an important rôle in transporting *mustard* from the atmosphere to the point of entry into the skin, and lends some support to the view that *mustard gas* passes down the sweat glands by a process in which surface phenomena play an important part.

There is a striking correspondence between the above results in which a water film facilitates the passage of *mustard gas* into

the tissues and the observation of Clowes, Perrott and Gordon that the passage of *mustard* through clothing is facilitated by the presence of from 3 to 5 per cent of water (4).

7. DISCUSSION

A variation in sensitivity of the skin of several hundred to one is observed in experiments with saturated vapor or paraffin oil solutions of dichloroethylsulfide. In experiments with different concentrations and times of exposure, using different species of animals, the variation in the susceptibility of the individuals of a given species to the effects of inhalation are of a very much lower order of magnitude—probably not more than five to one. Furthermore, on intramuscular or intravenous injection in dogs variations of no great order of magnitude have been observed (5). As has been discussed in a previous portion of the paper, it is evident that the differences in sensitivity observed are due to differences in the relative amounts absorbed compared to the amounts lost by evaporation. Hence, we feel justified in assuming that in individuals of the same species the amount of *mustard gas* required within the cell to produce pathological effects is roughly the same—that is the threshold concentration for the cell varies very little in different men.

In discussing the mechanism of absorption of *mustard gas* by the skin it is proposed to consider the problem from the standpoint of a three phase system in which—

A. The outer phase represents the external atmosphere containing varying concentrations of *mustard* vapors.

B. The middle phase represents the outer layers of the skin through which the *mustard* must pass in order to produce a toxic effect.

C. The inner phase represents the inner layer of the skin, particularly the protoplasmic contents of those cells of the tissues which are directly affected by the poison (see fig. 6).

For purposes of convenience A, B, and C in resistant individuals have been designated as A_n , B_n , and C_n and in sensitive individuals as A_s , B_s , and C_s .

If, as has been indicated above, the velocity with which *mustard* gas must pass into those reactive cells which have been designated as C in order to produce a given pathological effect is approximately constant in resistant and sensitive individuals—if the critical toxic threshold concentration in C_r is the same as in C_s —it necessarily follows that variations in concentration of gas or time of exposure in A required to produce equal effects in C_r and C_s must be attributable in great part to variation in the resistance offered by B_r and B_s to the passage of the gas.

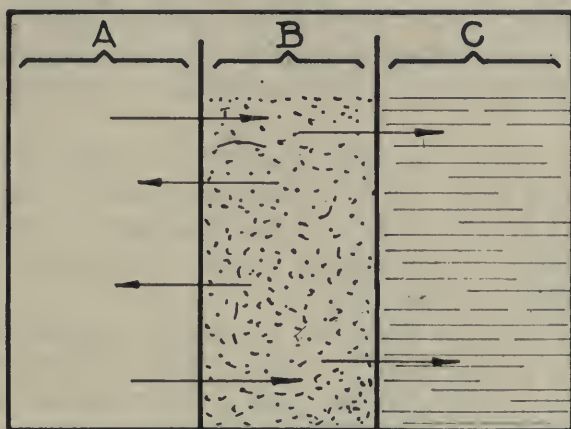


FIG. 6

The explanation for the difference in facility of passage through B_r and B_s may lie in one or more of the following causes:

1. Variation in facility of adsorption of *mustard* on interface between A and B.

2. Variation in the facility with which adsorbed *mustard* passes along gland surfaces by capillarity or into and through the epidermis by a process of diffusion of the adsorbed or dissolved gas.

3. Variation in the thickness of B_r and B_s or the actual distance to be traversed from A. to C.

4. Variation in the amount of gas required to saturate constituents of the skin which may be capable of adsorbing or

dissolving large quantities of the gas (for example, lipoids, pigment granules, etc. in negro and white skin).

5. Variation in the facility of passage of gas from B into C particularly from the lipoid non-aqueous phase into the aqueous phase of the protoplasm of those reactive cells in which the pathological effects are produced.

In the present stage of our knowledge it is difficult to determine the relative importance of the rôle played by each of these factors, but variation in the resistance of B may be conveniently represented pictorially either by varying its thickness, making B_r thicker than B_s , or by varying the angle of the average gradient of *mustard* concentration through B required to deliver a given amount of *mustard* in C; in which case that of B_r would be steeper than B_s .

It will readily be seen that in either case, a critical concentration of *mustard gas* would be delivered into C_s with greater facility than into C_r ; thus a shorter exposure or a lower initial concentration would be required to effect the passage into C_s of a reactive quantity of the poison.

Whatever mode of expression be adopted, the effect of evaporation after the exposure is evident. The concentration at the interface AB would be higher than at the interface BC, but it would rapidly decrease as a result of loss into the air.

The delivery of the necessary critical concentration of gas into C for a sufficient period of time to cause pathological changes would obviously depend upon the thickness of B, upon the angle of gradient of resistance to passage through B, upon the absolute quantity present in B and particularly upon the facility with which the concentration falls off at the interface AB as a result of evaporation. It appears desirable to utilize the available experimental data in an attempt to determine more specifically the relative importance of the individual factors enumerated above, viz.; surface adsorption, resistance to passage of the *gas*, thickness of skin, saturation capacity and threshold concentration required to produce toxic effects in C.

This last factor, i.e., threshold concentration in C—must be very low because (1) concentrations in the range of 0.002 to 0.005

mg. per liter represent not only critical points in the curves determined on the skin (page 7) but (2) also correspond very closely with the threshold concentrations observed in respiratory experiments, and (3) the enormous proportion of gas lost from the skin after exposure, as shown by capping experiments, indicates that the amount which is absorbed is very small. The extremely small amounts to which some individuals give a reaction is further evidence for this fact. This low threshold concentration in C leaves adsorption on the skin surface or one or more of the factors involved in the passage through B, as primarily responsible for the buffer effect exerted by the skin.

In the case of liquid burns or severe vapor burns the difference in the protective mechanism of resistant and sensitive skins appears to be reduced to a minimum; all the factors involved are probably saturated, whatever their capacity.

The observation that the difference between B_R and B_S is more and more accentuated with reduction in time of exposure or concentration points to surface adsorption as an important factor; reasoning in this direction is supported by the following facts:

1. Ten minutes after exposure of the skin to liquid *mustard gas* so large a proportion of the mustard may be removed by repeated washings with kerosene as to reduce the amount passing into the tissues below the critical level required to produce a blistering burn.

2. Washing with soap and water produces a corresponding effect but to a much less degree.

3. The presence of mustard gas on the skin surface may be demonstrated half an hour after exposure by transmitting a burn to another individual.

4. Since a 15-second exposure, may be raised by capping for an hour to the magnitude of a 3-minute exposure, the loss from the skin to the atmosphere must be far in excess of the amount passing into the interior. This suggests that the maximum concentration of mustard is on or near the surface.

5. The curves covering these capping reactions support the theory that surface adsorption is the factor of primary importance.

6. The fact that the skin may be left open to the atmosphere for a prolonged period, in some cases as much as 30 minutes to 45 minutes after exposure, and the intensity of the burn may still be raised by capping, indicates that loss of gas from the surface is still taking place.

7. The affinity of the skin of the resistant individual for mustard gas is far in excess of that of a sensitive one. The fact that B_R withdraws gas from B_s shows that B_R adsorbs gas more strongly than B_s and that the gas is still on or near the surface.

8. If exposures on resistant and sensitive skins have been so adjusted as to give equal burns if subsequently left uncovered, capping the exposures gives a worse burn in the resistant than in the sensitive case. This result corresponds with the fact that gas may be withdrawn by a resistant skin from a resistant skin longer than from a sensitive skin.

From the above considerations it seems probable that difference in the adsorptive capacity of the skin surface is the most important limiting factor in determining the degree of exposure in A necessary to effect the delivery of a toxic concentration in C.

In considering the relative importance of factors other than surface adsorption on B and threshold concentration in C, it is obvious that the greater resistance of the negro's skin as compared with the white's skin may reasonably be attributed in part at least, to differences in actual thickness of the skin, to fats and lipoids which appear to be more plentiful and to pigments which are known to be more plentiful, in the negro skin than in the white.

The very short minimum time within which the first reactions become visible in certain sensitive individuals would seem to indicate that the first traces passed through the skin fairly rapidly (1).

The experiments with water films seem to support the view that mustard passes into the sweat glands from which it is adsorbed after passing by capillarity to areas adjacent to the cells of the underlying tissues in which pathological effects are produced.

The capping experiments support this view. The passage of *mustard* into the sweat glands would obviously be facilitated by maintaining the vapor pressure.

These experiments should be considered in the light of certain observations concerning the absorption of dichloroethylsulfide in other parts of the body.

Even though *mustard gas* is very rapidly hydrolyzed at body temperature, inhalation of high concentrations of the vapor, intravenous injection of large quantities of the saturated aqueous solution and application of the oil to the skin cause not only local lesions but also marked and characteristic systemic effects (5).

It is obvious, therefore, that after its introduction into the blood stream a portion at least of the unhydrolyzed material must be taken up by lipoidal constituents and thus protected from immediate hydrolysis. After intradermal, subcutaneous, intraperitoneal or intramuscular injections of this substance the severe necrotic lesions which might be expected from its action are not observed. The pathological changes consists largely in the development of intense edema at the site of injection, with the characteristic effects on the gut and adrenals. It is possible that, when it is thus injected directly into the deeper tissues which are well supplied with blood, and which lack the non-aqueous binder of the skin, it is rapidly absorbed without doing much local damage. Moreover, the bountiful blood supply would go far to counteract the intracellular liberation of acid, and would thus circumvent its local toxic action. This suggestion is supported by the pathological changes observed in the respiratory tract in animals gassed with *mustard*. Though congestion and necrotic sluffing appear in the upper tract, the lower tract is practically undamaged until very high concentrations are used, when some edema may appear. Since the lower respiratory tract is primarily an absorbing tissue, it is in very close association with the blood stream. The absorbed poison might be rapidly removed, and such hydrolysis as would inevitably occur might be counteracted by neutralization in the buffer system of the blood.

In considering the toxic action of a series of war gases it is observed that there is marked contrast in the pathological effects exerted upon the upper and lower respiratory tract and upon the skin.

There appears to be a certain relation between the areas exhibiting maximum pathological changes and certain physical and chemical properties of the gas, for example, vapor pressure, lipid-water distribution coefficient and rate of hydrolysis in water.

Phosgene and superpalite, which have a very high vapor pressure and which hydrolyze very rapidly on contact with water, exert a very destructive effect on the lower respiratory tract, but do not markedly affect the upper tract or irritate the skin.

It is obvious that under given condition of vapor pressure, lipid solubility etc., *a given amount of gas finds its way into the cell in a given time and if the rate of transportation through the cell is very rapid, the question as to whether or not a toxic concentration of acid will be produced within the cell depends upon the rate of hydrolysis, or the relation between transportation velocity and rate of hydrolysis.*

A low lipid-water partition coefficient should facilitate the decomposition of gas within the cell by raising the concentration in the water phase and by diminishing the proportion removed by the lipid constituents of the blood.

The failure of such volatile substances as phosgene and superpalite to exert any effect on the skin is readily explained in the light of experiments with *mustard*, the loss of which from the skin to the air has been demonstrated to be very great in spite of its relatively low vapor pressure.

Substances like *mustard gas* with a low vapor pressure and which hydrolyze comparatively slowly, would, after their absorption by the lower respiratory tract, have an opportunity to pass into the lipoidal phases of the blood before hydrolysis reached such dimensions within the cell as to cause pathological effects.

But in the cells of the upper bronchi and the skin, where transportation is comparatively insignificant, an accumulation would

result, and ultimately sufficient acid would be liberated to produce toxic effects.

Phenyldichloroarsine and ethyldichloroarsine, with a low vapor pressure (resembling mustard gas somewhat in their physical properties) but having a high rate of hydrolysis attack the skin and upper respiratory tract just as mustard gas does, and cause profuse edema in the lower respiratory tract just as do phosgene and superpalite.

Unfortunately, these investigations were incomplete when the suspension of hostilities brought an end to experimental work.

Consequently, though a large amount of additional data is available suggesting a relation between the physico-chemical and pharmacological properties of substances which played a rôle in chemical warfare, it does not appear advisable to attempt further generalization at the present stage of our knowledge.

SUMMARY

I. The experimental data indicate—

1. That *mustard gas* is first adsorbed by some element on or immediately adjacent to the skin surface.

2. That while a portion of the *mustard* passes rapidly inward to a point from which it cannot subsequently be removed the greater portion remains on or near the surface for a considerable period, a proof of which is that it may be removed even after 10 or 15 minutes by persistent washing with organic solvents.

3. That the amount of *mustard* passing into the atmosphere from an exposed surface far exceeds the amount passing into the inner strata of the skin. This loss is very great at first and is still demonstrable after 45 minutes.

4. That the time of exposure necessary to produce a positive reaction bears a definite relation to concentration and varies for different individuals.

5. That a resistant skin adsorbs far more gas than a sensitive skin, and gas may be withdrawn from the latter by the former. That difference in sensitivity of different skins is due principally to difference in saturation adsorptive capacity.

6. That the *intracellular threshold concentration* of gas required to produce pathological changes in the skin is approximately the same in resistant and sensitive individuals.

II. Variations in the lipoid solubility, vapor pressure and rate of hydrolysis of various war gases in relation to variations in their toxic action have been tentatively discussed from a physico-chemical standpoint.

In conclusion, we desire to thank Messrs. J. W. Williams and P. S. Barba for aid in carrying out certain of the experiments, and also to thank the numerous subjects who subjected themselves to experimental burns.

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CONCERNING THE ACTION OF LOCAL ANESTHETICS ON STRIATED MUSCLE

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INTRODUCTION

The action of local anesthetics on skeletal muscle has been the subject of remarkably little investigation. This is especially surprising in view of the fact that coca leaves have been used and are still used by the natives (1) in Peru and Bolivia to relieve fatigue and to stimulate muscular work. The action of cocain upon skeletal muscle has been studied by a number of observers, but among these there is no unanimity of opinion on the subject. Thus, Mosso (2) claimed that small doses of cocain exert a stimulating effect on striated muscle, while Anrep (3), Kobert (4), Freund (5), Berthold (6), Alms, (7), Feinberg (8), and Sigheicelli (9) could not observe any such effect and noted either a depression or no action at all. In the present research the authors have undertaken a study of a number of local anesthetics in respect to their effect on the irritability and contraction of striated muscle.

SUBSTANCES STUDIED

The following drugs were employed in the present investigation:

- (1) Cocain hydrochloride or the hydrochloride of benzoylmethylecgonin.
- (2) Novocain nitrate or the nitrate of aminobenzoyl diethylamino ethane.
- (3) Alypin or the hydrochloride of benzoxy-dimethylaminomethyl-dimethyl-amino-butane.
- (4) Stovain or the hydrochloride of benzoxy-methyl-dimethyl-amino-butane.

(5) Beta eucain or the hydrochloride of trimethyl-benzoxyl-oxyperidine.

(6) Holocain hydrochloride or the hydrochloride of phenetidyl-acetphenetidin.

(7) Phenmethylool or benzyl alcohol—the new anesthetic recently described by Macht (10).

(8) Allocain S. or the hydrochloride of benzoyl diethyl mudriatin.

(9) Apothesine—the cinnamic ester of gammadiethylamino propyl alcohol.

These substances were dissolved in Ringer's solution in various concentrations.

METHOD

The work was carried on during the months of June and July, 1918. The action of the drugs was studied on the muscles of a cold-blooded animal, the frog, and of a warm-blooded animal, the rat. The muscle studied in most cases was the gastrocnemius. The effect of a drug on the muscle was exerted by immersing the excised muscle preparation in a solution of the substance and not by first injecting the animal with the drug and subsequently excising the muscle. This point is of special importance in connection with the results obtained as will be explained later in the paper.

The following physiological properties of striated muscle were studied: first, its excitability by the electric current; second, the form of its contraction curve; and third, the fatigue curve.

The excitability of the excised frog's gastrocnemius was determined by immersing the preparation in the solution of a drug, and then by stimulating the muscle directly with the electrical current every five minutes. At each five minute interval, the strength of the stimulus required to produce a contraction was noted and written down. The excitability of the rat's muscle was studied in the same way, except, that in this case the preparation was immersed in a warm oxygenated medium.

The contraction curves of both the frog's and the rat's muscles were inscribed in the usual manner of producing myogram records.

The fatigue curves were obtained by stimulating the muscles successively with electric shocks at intervals of one or two seconds.

In every experiment two muscles from the two sides of the body were employed; one, in order to study the effect of the drug, and the other as a control. Experiments were also made on muscles which were previously treated with curare. It may be here stated at once that in every case the curarized and non-curarized preparations gave the same results, so far as the effect of the drug was concerned. In a number of experiments, the muscles were also studied by injecting the whole animal, and subsequently excising the gastrocnemius.

THE EFFECT OF LOCAL ANESTHETICS ON THE EXCITABILITY OF SKELETAL MUSCLE

The excitability of a frog's gastrocnemius muscle soaked in a 1 per cent solution of a local anesthetic diminishes gradually and is finally lost completely. The time required for the paralysis to set in depends on the kind and dose of the drug studied. In the present investigation it was found that phenmethylo, allocain S., beta eucain, and stovain act more quickly than cocain, novocain, alypin, holocain, and apothesine; the first group taking from twenty to thirty-five minutes to paralyze the muscle, while the latter group did not produce that effect until much later—from fifty to one hundred and thirty minutes. Tables 1 and 2 will illustrate the action of the various anesthetics on the excitability of the frog's gastrocnemius muscle.

The same experiments made with curarized muscles gave similar results, except that the excitability of the muscle, after treatment with curare, was lower than normal to start with. The muscles in all cases after having been paralyzed by local anesthetics could not be resuscitated by washing with Ringer's solution.

In the experiments with muscles of the rat, the same results qualitatively were obtained as in the case of the frog. The rat's muscles, however, were more sensitive to weaker solutions of

the drugs used and were paralyzed more quickly. Table 3 will suffice to illustrate the behavior of a rat's gastrocnemius in a 0.5 solution of the anesthetics used.

TABLE 1

The change in excitability of frog's gastrocnemius in a 1 per cent solution of locally anesthetic substances. Coil distance in cm.

TIME	COCAIN HY- DROCHLORIDE		NOVOCAIN NITRATE		ALYPIN		HOLOCAIN HY- DROCHLORIDE		APOTHESINE	
	Left (Con- trol)	Right	Left (Con- trol)	Right	Left (Con- trol)	Right	Left (Con- trol)	Right	Left (Con- trol)	Right
Before immersing.....	26.2	28.2	27.5	28.7	22.5	25.5	19.0	20.5	19.5	25.0
After 5 minutes.....	25.5	24.5	27.5	22.5	22.5	23.0	18.5	20.0	19.5	23.0
After 10 minutes.....		25.0		21.5		19.5		17.5		22.0
After 15 minutes.....	25.5	23.0	27.2	20.0	22.0	15.3	18.5	16.0	19.5	19.5
After 20 minutes.....		20.0		18.0		8.5		15.5		17.5
After 25 minutes.....	25.3	12.2	27.5	9.2	21.8	8.5	18.0	15.5	19.0	16.0
After 30 minutes.....		9.8		8.4		7.5		15.0		9.0
After 35 minutes.....	25.4	10.0		8.0	21.7	7.5	18.3	10.0	19.0	8.5
After 40 minutes.....		9.4	27.3	7.5		7.5		7.5		7.0
After 45 minutes.....	25.0	9.0		7.5	21.8	6.4	18.0		19.0	6.0
After 50 minutes.....		7.0	27.0	7.7		4.0				0
After 55 minutes.....	25.0	6.0		7.5	21.5	0	18.0	4.5	18.5	0
After 60 minutes.....	24.5	0	27.0	6.4		0		2.7	18.5	0
After 70 minutes.....	24.0	0		6.5	21.0	0	18.0	0		
After 80 minutes.....	24.0	0	27.0	6.0				0		
After 90 minutes.....				6.0			17.5	0		
After 120 minutes.....			26.5	2.0						
After 130 minutes.....			26.5	0						
After 140 minutes.....			26.0	0						

TABLE 2

TIME	PHENMETHYLOL		B. EUCAIN		ALLOCAIN S.		STOVAIN	
	Left (Control)	Right	Left (Control)	Right	Left (Control)	Right	Left (Control)	Right
Before immersing.....	22.5	21.0	22.5	29.3	28.5	27.0	24.7	26.0
After 5 minutes.....	22.5	15.5	22.5	25.5	28.5	26.0	24.5	17.0
After 10 minutes.....		15.0		21.0	28.0	21.0		13.8
After 15 minutes.....	28.2	6.5	22.2	17.8	28.0	7.7	24.5	7.5
After 20 minutes.....		0		9.0		4.5		6.5
After 25 minutes.....	22.0	0	22.0	4.0	28.0	0	24.0	2.5
After 30 minutes.....	22.0	0		2.0	27.5	0	24.0	0
After 35 minutes.....			22.0	0	27.5	0	24.0	0
After 40 minutes.....			22.0	0				
After 45 minutes.....			22.0	0				

TABLE 3

The change in the excitability of rat's gastrocnemius in a 0.5 per cent solution of local anesthetics. The figures indicate the distance of the secondary coil in cm.

TIME	COCAIN HYDROCHLORIDE		PHENMETHYLOL		ALLOCAIN S.	S. M. C.
	Left (Control)	Right	Right (Control)	Left	Left (Control)	Right
Before immersing.....	11.0	12.0	8.5	8.5	9.0	9.5
After 5 minutes.....	11.0	9.0	8.5	6.3	8.5	7.0
After 10 minutes.....	10.5	7.8	8.5	1.0	8.5	2.0
After 15 minutes.....	10.5	6.5	8.5	0	7.5	0
After 20 minutes.....	9.3	2.0	8.5	0	7.5	0
After 25 minutes.....	9.0	0	8.5	0	7.5	0
After 30 minutes.....	8.5	0				
After 35 minutes.....	8.5	0				

ANALYSIS OF THE CONTRACTION CURVES¹

Myograms of single contractions produced by the break-shock were made in the usual manner. These curves showed the latent period, and the contraction and relaxation periods of the muscles

¹ Owing to expense of reproduction, only a few curves can be here given as illustrations.

in normal preparations and in those treated with the local anesthetics. The results are illustrated by figures 1 and 2, and by table 4.

It was noted that the chief effect of all the local anesthetics was to diminish the height or strength of the contractions, the dim-



FIG. 1. THE CONTRACTION CURVE OF FROG'S GASTROCNEMIUS (CURARIZED)

Aa, Right, after immersing for 10 minutes in a 1 per cent solution of cocain hydrochloride. Ab, Left, control. Time maker, 1/100 second. Magnification, 8 times. Counterweight, 10 grams.

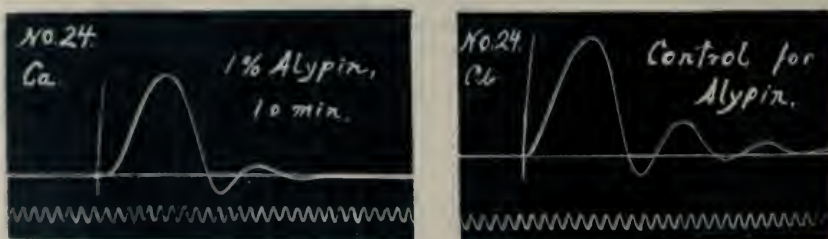


FIG. 2. THE CONTRACTION CURVE OF FROG'S GASTROCNEMIUS (CURARIZED)

Ca, Right, after immersing for 10 minutes in a 1 per cent aлыпin solution. Cb, Left, control. Time maker: 1/100 second. Magnification, 8 times. Load, 10 grams.

inution amounting to about 75 per cent of the original height after treatment with the drug for ten minutes in some cases, and in no case was there any increase in contraction observed. The latent period was slightly prolonged in many cases. The contraction and the relaxation periods were generally but little affected.

TABLE 4

The strength of contraction, latent period, contraction and relaxation periods of the frog's gastrocnemius immersed in a 1 per cent solution of a local anesthetic

SUBSTANCE	SIDE	STRENGTH OF MAX. CONTRAC- TION	LATENT PERIOD	CONTRAC- TION PERIOD	RELAXA- TION PERIOD
		<i>mm.</i>	<i>seconds</i>	<i>seconds</i>	<i>seconds</i>
Cocainhydrochloride...	Left (control)....	25.0	0.007	0.043	0.052
	Right.....	18.0	0.008	0.046	0.058
Novocain nitrate.....	Right (control)..	35.0	0.011	0.044	0.057
	Left.....	23.0	0.009	0.045	0.060
Alypin.....	Left (control)....	34.0	0.008	0.049	0.052
	Right.....	20.5	0.005	0.051	0.062
Holocain hydrochloride	Right (control)..	27.0	0.008	0.047	0.047
	Left.....	12.0	0.010	0.048	0.062
Apothesine.....	Left (control)....	27.0	0.011	0.050	0.051
	Right.....	20.0	0.007	0.049	0.058
PhenmethyloI.....	Right (control)..	32.0	0.013	0.040	0.045
	Left.....	7.0	0.007	0.050	0.059
Allocain S.....	Left (control)....	28.0	0.008	0.047	0.055
	Right.....	21.0	0.008	0.052	0.060
B. eucain.....	Right (control)..	28.0	0.008	0.048	0.052
	Left.....	15.5	0.005	0.055	0.056
Stovain.....	Right (control)..	34.5	0.011	0.049	0.060
	Left.....	21.5	0.009	0.055	0.059

EFFECT ON THE FATIGUE CURVE

This was studied by repeated stimulation of the muscle preparations with electrical shocks at intervals of one or two seconds. In every experiment a control test was made on the corresponding normal muscle from the other side of the same animal. It was found that all the local anesthetics produced fatigue more quickly in both frogs and rats. The results are illustrated by figures 4 and 5. An estimate of the total amount of work performed by the muscles was obtained by determining the average height of



FIG. 3. THE CONTRACTION AND FATIGUE CURVES OF RAT'S GASTROCNEMIUS

Ha and *Hc*, Right, after 10 minutes' immersion in a 0.5 per cent phenethylol solution at room temperature. *Hb* and *Hd*, Left, control. Magnification, 8 times. Load, 10 grams. Time maker, 1/100 second. Stimuli, 1 per 2 seconds. Strength of contractions *Ha*, 28; *o*, *Hb*, 39.0 mm. Latent period, *Ha*, 0.009; *Hb*, 0.008 second contraction period, *Ha*, 0.050, *Hb*, 0.051. Relaxation period, *Ha*, 0.059; *Hb*, 0.063 second. Total number of contractions or total working time in seconds, *Hc*, 740; *Hd*, 1206. Total amount of work in gramcentimeters, *Hc*, 6240; *Hd*, 8550. Percentage of decrease, 27.

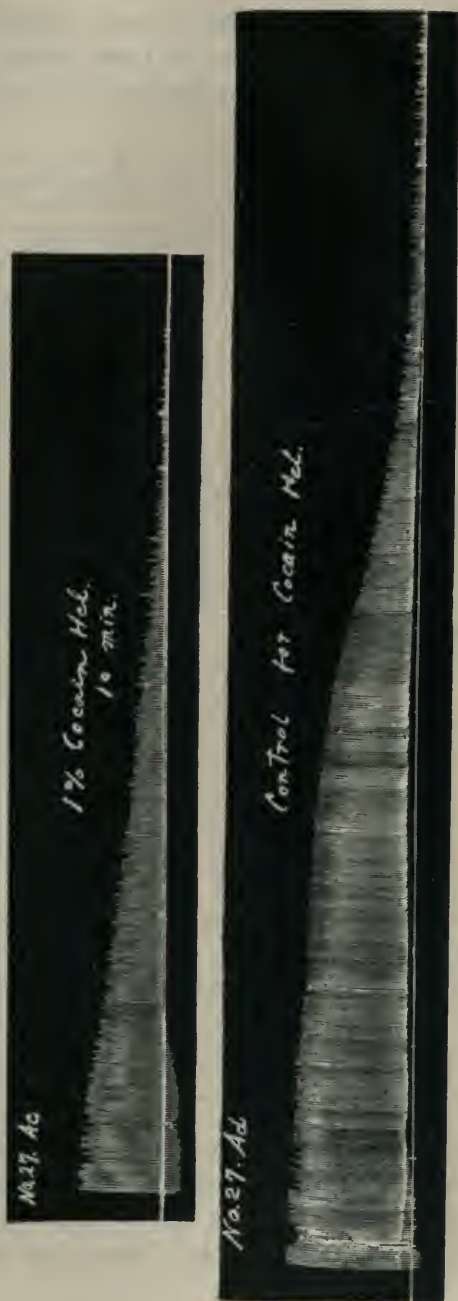


FIG. 4. THE FATIGUE CURVE OF FROG'S GASTROCNEMIUS (CURARIZED)

Ac, Right, after 10 minutes' immersion in a 1 per cent cocaine hydrochloride solution. Ad, Left, control. Magnification, 8 times. Load, 10 grams. Stimuli, 1 per second. Total number of contractions or total working time in seconds, Ac, 352; Ad, 566. Total amount of work in gramcentimeters, Ac, 3255; Ad, 8165. Percentage of decrease, 60.

the contractions and the period of fatigue, and multiplying it by the counterweight, which was 10 grams in most cases. It was found that the total amount of work performed by the muscles

TABLE 5
The effect of local anesthetics on the total amount of muscle work

SUBSTANCE	SIDE	TOTAL NUMBER OF CONTRAC- TIONS OR TOTAL WORKING TIME (SECONDS)	TOTAL AMOUNT OF MUSCLE WORK (GRAMCENTI- METERS)	PERCENTAGE OF DECREASE IN MUSCLE WORK
				<i>per cent</i>
Cocain hydrochlorid.....	Left control)....	566	8163	60
	Right.....	352	3255	
Novocain nitrate.....	Right control)...	557	6295	40
	Left.....	377	3754	
Alypin.....	Right (control)..	355	4333	65
	Left.....	318	1533	
Holocain hydrochloride...	Right (control)..	480	5275	62
	Left.....	282	2025	
Apothesine.....	Left (control)....	692	8497	66
	Right.....	367	2884	
PhenmethyloI.....	Right (control)..	615	6412	74
	Left.....	345	1675	
Allocain S.....	Left (control)....	650	8050	54
	Right.....	410	3710	
B. eucaïn.....	Right (control)..	565	7355	69
	Left.....	272	2312	
Stovain.....	Right (control)..	542	7810	55
	Left.....	357	3530	

from the beginning of stimulation to the onset of complete fatigue was greatly diminished after treatment with local anesthetics, the diminution varying in degree from 40 to 74 per cent (see table 5). In the case of the rat's muscles, fatigue was produced

more quickly than in the case of the frog's muscles. It was noted that the onset of fatigue was rather slow after cocain and novocain, and very rapid after phenmethylo and allocain S.

In connection with the above experiments, a few observations were made on the effect of dilute hydrocyanic acid on the contractions of skeletal muscle, inasmuch as this drug is known to have a local anesthetic action also. The preparations were immersed in 0.25 per cent hydrocyanic acid solution. It was found that the excitability of the frog's gastrocnemius in such cases was completely lost within fifty minutes. The contraction and fatigue curves were also very greatly impaired. More concentrated solutions produced a more rapid and more toxic effect.

ACTION OF MINIMAL DOSES

In view of the fact that Mosso claimed that small doses of cocain produce a primary stimulation of the contractions of the skeletal muscle, the authors made experiments on the effect of very weak solutions of cocain and the other local anesthetics studied. It was found that neither 0.5 per cent, nor 0.1 per cent, nor 0.05 per cent, nor even 0.01 per cent caused any primary increase in the strength of the contractions. The effect of small doses of the other local anesthetics also showed no primary stage of stimulation.

ANALYSIS OF THE COCAIN ACTION

As is well known cocain can be split into three constituents: ecgonin, methyl alcohol, and benzoic acid: it is also a familiar fact to all pharmacologists that the local anesthetic action of cocain requires a combination of these three constituents, and cannot be produced by ecgonin alone. It was therefore interesting to analyze the cocain effect on skeletal muscle a little more carefully, and to inquire into the action on such muscle of its individual products of decomposition. Accordingly, experiments were made with a 1 per cent solution each of ecgonin hydrochloride, methyl alcohol, and sodium benzoate, and with various mixtures of the same. It was found that ecgonin hydrochloride acts

as a powerful depressant of the skeletal muscle, while methyl alcohol and sodium benzoate produced but a very slight effect on that tissue. Furthermore, it was found that benzoyl ecgonin was much less depressant than ecgonin itself.

A mixture of ecgonin hydrochloride, sodium benzoate, and methyl alcohol, in the proportions in which they are found in cocain, was found to produce a gradual depression of the excitability and contractions of the muscles. On the other hand, a mixture of benzoyl ecgonin and methyl alcohol was found to be

TABLE 6

The change in excitability of the frog's gastrocnemius (curarized) in solutions of cocain decomposition products

TIME	1 PER CENT ECGONIN DROCH.	1 PER CENT SODIUM BEN- ZOATE	1 PER CENT BENZOYL EC- GONIN	1 PER CENT METHYL AL- COHOL	MIXTURE (b) ECGONIN, SOD. BENZ. METHYL ALCOHOL	MIXTURE (c) BENZOYL EC- GONIN, METHYL ALCOHOL	1 PER CENT TROPIN HCl	CONTROL
	Left	Right	Left	Right	Left	Right	Right	Left
Before immersing.....	12.0	13.0	11.5	15.0	14.0	13.0	11.0	12.0
After 5 minutes.....	10.8	13.0	11.0	15.0	14.0	13.0	10.5	12.0
After 10 minutes.....	10.0	13.0	10.8	14.5	11.5	13.0	9.8	12.0
After 15 minutes.....	10.0	12.5	11.0	14.5	9.5	13.0	10.0	12.0
After 20 minutes.....	9.0	12.5	11.0	15.0	7.7	13.0	10.0	11.5
After 25 minutes.....	7.5	12.5	11.0	14.5	7.0	13.0	9.5	11.5
After 30 minutes.....	5.0	12.5	11.0	14.5	7.5	12.5	7.5	11.5
After 35 minutes.....	5.0	12.5	10.5	14.5	5.0	12.5	5.0	11.5
After 40 minutes.....	4.0	12.5	10.0		3.8	12.5	0	11.0
After 45 minutes.....	3.0	12.5	10.5		3.5	12.5	0	11.0
After 50 minutes.....	0	12.0						
After 55 minutes.....	0	12.0						
After 60 minutes.....	0	12.0						

very little toxic. The slightly depressant action of benzoyl ecgonin, found by the authors, agreed well with the observations of Stockman on its action on frogs' muscles (11). The toxicity of benzoyl ecgonin was found to be much less, however, than that of ecgonin alone. It was, furthermore, interesting to find that tropin, which is very closely related to ecgonin was also a very marked depressant for skeletal muscle. Table 6 illustrates some of the analytical data.

DISCUSSION

From the above described experiments, it was found that not only cocain, but all the local anesthetics examined, produced depression of striated or skeletal muscle. Inasmuch as all the experiments on isolated muscle preparations were also repeated on curarized specimens, it follows that this depressive action is to be ascribed to an effect directly on the muscle itself. Even with very small doses of cocain, there was always found a depression, and in no case was there any primary stimulant action noted. It would therefore seem that stimulating effects of coca leaves, as employed by the South American natives, must be due entirely to the central action of cocain, and not to its peripheral effect. The present authors have found a much greater depression produced on skeletal muscle than that described by some other observers. This difference can be easily explained, inasmuch as all the experiments in the present investigation have been made on excised muscle preparations which were immersed in solutions of the drug; while in the experiments performed by other observers, the frogs were first injected with cocain and the muscles were excised afterwards. The present authors have found that when a dose of cocain is injected into a frog a slowing of the heart, and even a complete stoppage of that organ, quickly follows. They have furthermore observed that not only the heart itself is paralyzed by cocain, but the lymph hearts are also paralyzed by that drug. It follows, therefore, that when a dose of cocain is injected into a frog both the systemic and the lymphatic circulation is inhibited and the drug is very poorly distributed through muscular tissue. It is for this reason that the muscle preparations in such cases show less depression than when the drugs are directly applied to them as in the present research. The effect on rat's muscles was qualitatively the same as on frogs. This is of especial interest, as no studies on warm blooded muscles have been made before by other authors in this connection.

SUMMARY

1. The action of a large number of anesthetics was studied on excised skeletal muscles of the frog and the rat.

2. It was found that all of the substances studied depressed the excitability and the contractility of striated muscle, and caused a quicker onset of fatigue.

3. No primary stimulating effect was noted after the exhibition of even very small doses of cocain or other local anesthetics.

4. Of the decomposition products of cocain, ecgonin was found to be a powerful depressant, while benzoyl ecgonin was only very slightly depressant in its action, and sodium benzoate and methylalcohol produced practically no effect.

5. The stimulating effect of cocain on muscular work cannot, therefore, be due to its peripheral action on the muscles themselves, but must be ascribed to a central action.

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ADENINE MONONUCLEOTIDE¹

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When a neutral or faintly alkaline solution of nucleic acid is treated with potassium permanganate, its various groups are destroyed in a definite order, leaving an undecomposed residue of the nucleic acid, whose chemical isolation offers little difficulty; and the extent to which this destruction of the nucleic acid will proceed can be closely controlled by the amount of permanganate used. At the same time the permanganate is quantitatively reduced to insoluble manganese dioxide hydrate which can be removed by filtration, so that the process offers a very advantageous method of examining a series of more or less resistant, larger or smaller portions of the nucleic acid molecule.

By repeated trials it was found that a very definite stage of the oxidation occurs when the nucleic acid and the permanganate are used in equal amounts, and we will confine ourselves for the present to this particular phase of the reaction.

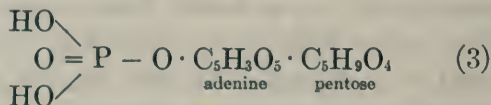
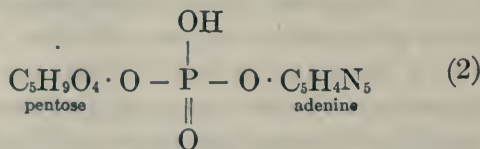
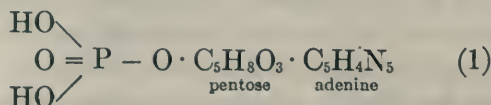
Of the four nitrogenous groups of nucleic acid, three are destroyed under the conditions stated. Neither cytosine, uracil nor guanine was obtained by acid hydrolysis of a large amount of the oxidation product. But the fourth group (adenine) is not cut off from its nucleotide linkage nor even deaminized; for adenine mononucleotide can be isolated from the products of the oxidation in the form of crystalline needles which may be purified to any desired extent by repeated crystallization from hot water.

The substance yields a reducing carbohydrate, responds to the pentose color reactions and has the chemical composition

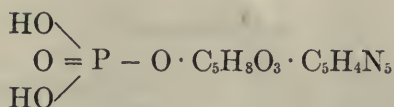
¹ The essentials of this paper were published without experimental data. *The Journal of Pharmacology and Experimental Therapeutics*, 1918, xii, 253.

required for the formula $C_{10}H_{14}N_5PO_7 \cdot H_2O$. By mild acid hydrolysis, it yields both phosphoric acid and adenine in the amount required for adenine mononucleotide but does not produce guanine, cytosine nor uracil. There seems no escape from the conclusion that the substance is composed exclusively of three groups (adenine, phosphoric acid and pentose) and the following considerations will show the order in which these three groups are arranged.

Three groups can be arranged in three and only three different orders. That is to say any one of the three may be the central one connecting the other two as is shown in the following scheme.



Adenine mononucleotide is a dibasic acid as we have found both by titrating a known weight of the substance with a standard solution of sodium hydroxide and by analysis of its crystalline brucine salt which contains two equivalents of brucine. Hence formula (2) above, being the formula of a monobasic acid, is excluded. The phosphoric acid group cannot be the central group. Again, in formula (3) the adenine group is internal and therefore adenine could not be set free faster than phosphoric acid. But by mild acid hydrolysis of adenine mononucleotide, adenine is liberated very much faster than phosphoric acid. Hence the adenine group cannot be central and formula (3) is excluded. There remains formula (1)



which is in accordance with everything that we know of the substance.

A most interesting matter came to light in a study of the rate at which phosphoric acid is set free from this nucleotide. It may be recalled that similar studies have been made with yeast nucleic acid and with other nucleotides of simpler constitution.² To explain the results of these experiments it was found necessary and sufficient to assume that phosphoric acid is set free from nucleotides according to two widely different laws. That the two purine nucleotides conform to one of these laws while the two pyrimidine nucleotides conform to the other. This view of the matter has been found satisfactory to the extent that it is possible to tell with a fair degree of accuracy either in a mixture of nucleotides or in compound nucleotides, what fraction of the material is purine nucleotide by ascertaining the rate at which phosphoric acid is set free from the material by acid hydrolysis.

The possession of pure adenine mononucleotide furnishes us a means of experimentally testing one of the assumptions stated above; and it was found that the substance gives off its phosphoric acid in accordance with the "purine law."

It may be stated that the matters here referred to would not likely be true if any of the compound or mixed nucleotides possessed anywhere a nucleotide linkage through their phosphoric acid group. Each of the mononucleotides has its own phosphoric acid law which applies to the nucleotide when it is joined to other nucleotides just as though no such union existed.

A detailed discussion of these matters and of the constitution of yeast nucleic acid will appear later.

² Jones and Riley, *Journ. Biol. Chem.*, xxiv, proc., 1. Jones and Read, *ibid.*, xxix, 123; xxxi, 39; xxxi, 337.

EXPERIMENTAL

Oxidation of yeast nucleic acid with potassium permanganate and preparation of the brucine salt of the product. A solution of 25 grams of yeast nucleic acid in 250 cc. of hot water with a few drops of caustic soda, was treated with 250 cc. of hot aqueous solution containing 25 grams of potassium permanganate. The solutions should not be boiling hot and the permanganate must be added in small portions at a time in order to avoid the occurrence of a violent reaction with evolution of gas. This phenomenon is very liable to happen but it will not occur if the conditions stated are observed.

After all the permanganate had been added the product was warmed until the manganese dioxide separated sharply leaving a perfectly clear pale yellow fluid. This was filtered with a pump and the black cake of manganese dioxide was extracted twice by grinding with 250 cc. of boiling water each time. The united filtrates were made faintly acid with acetic acid, evaporated to a syrup at 50° under diminished pressure and hardened to a pale yellow dry powder with absolute alcohol.

In order to test the product for combined cytosine and uracil, 50 grams of the powder were hydrolyzed with sulphuric acid and the product was examined in the ordinary way. Uracil was not present. Cytosine was not found, but the method of isolating cytosine from such a mixture is so unsatisfactory that the substance may have escaped detection. The absence of any trace of guanine was easily shown.

The dry powder that had been hardened with alcohol was dissolved in water and faintly acidified with acetic acid, preparatory to the precipitation of the lead salt with acetate. But it is not necessary to dry the substance at all for the subsequent work. As a rule the following procedure was employed. The fluid obtained from 100 grams of nucleic acid, after filtering off the manganese dioxide, was evaporated at 50° to about 200 cc. and treated at the boiling point with 200 cc. of 25 per cent neutral lead acetate. After the fluid had become cool, 100 cc. of 10 per cent silver nitrate were added and the precipitated mixture of

silver and lead salts was filtered off, suspended in hot water and decomposed with hydrogen sulphide. The excess of the gas was boiled out of the filtrate from lead-silver sulphide and the hot fluid was treated with lead acetate in slight excess. This second precipitation of the lead salt can be done sharply and its employment very much improves the product.

The lead compound was decomposed as before. The solution obtained was evaporated under diminished pressure to about 100 cc. and neutralized to litmus in the hot with a concentrated alcoholic solution of brucine. On cooling, the fluid stiffened to a mass of crystalline brucine salt which was crystallized once from hot water (to remove any trace of nucleotide that may have escaped neutralization) and then ten times out of 35 per cent alcohol. This last procedure will remove any brucine that may have been used in excess, and by the occasional use of animal charcoal the recrystallization leads finally to a perfectly colorless product consisting of feathery needles which melt at 173 to 174°.

Preparation of adenine nucleotide from the brucine salt. The brucine salt was suspended in 25 times its weight of boiling hot water (in which amount of water the substance does not completely dissolve) and made very faintly alkaline to litmus with ammonia. The suspended brucine salt goes completely into solution but no brucine separates. As the solution cools the careful addition of ammonia will throw out brucine in crystals that can be filtered easily.

The filtrate from brucine was extracted twice with small portions of chloroform, acidified faintly with acetic acid, concentrated under diminished pressure at 45° and treated with a slight excess of lead acetate. The lead compound was suspended in water, decomposed with hydrogen sulphide and evaporated to a small volume at 45° under diminished pressure. On standing over night the concentrated solution deposited a mass of crystalline needles which upon recrystallization from hot water, formed long transparent colorless rods.

Solutions of the nucleotide exhibit supersaturation to a remarkable degree. When a portion of the substance is dissolved

in the smallest possible amount of boiling water, the solution may stand for several hours after cooling without depositing any crystals, but in a sufficient time (over night), practically all of the nucleotide crystallizes.

Frequent recrystallization from hot water somewhat decreases the solubility of the nucleotide, although its chemical composition is not thereby changed.

Properties and chemical composition of the nucleotide. As already stated, the substance crystallizes from hot water with a molecule of water of crystallization. This is lost with considerable difficulty at 115° but more rapidly between 125° and 130°. Upon exposure to the air, the anhydrous substance takes up its water of crystallization quantitatively. Finely crystalline specimens of the nucleotide are occasionally obtained whose loss in weight at 125° falls somewhat below the amount required for a molecule of water. These dried specimens always give an exact nitrogen analysis for the anhydrous nucleotide and on exposure to the air, take up not a molecule of water, but the amount of water that they had lost by heating. It is thus possible that crystals of the hydrous nucleotide occasionally contain a small amount of an anhydrous substance.

The crystalline nucleotide almost uniformly gives dehydration figures like the following:

- I. 0.3010 lost 0.0147 at 125° (4.88 per cent instead of 4.90 per cent). On exposure to the air for twelve hours, the substance regained 0.0139.
- II. 0.2874 lost 0.0140 at 125° (4.8 per cent instead of 4.90 per cent). On exposure to the air for twelve hours the substance regained 0.0133.

The following is one of three exceptional cases: 0.2402 lost 0.0105 at 120° (4.37 per cent instead of 4.90 per cent). On exposure to the air for twelve hours, the substance regained 0.0104.

The nucleotide in most minute amount responds to the pentose color reactions both with hydrochloric acid and orcin, and with hydrochloric acid and phloroglucine. By hydrolysis with 10 per cent sulphuric acid it forms a reducing carbohydrate but no guanine.

Five hundred mgm. of the substance were heated with 10 cc. of 10 per cent sulphuric acid for two hours at 100°. The product was divided into two portions. One portion was made alkaline with ammonia but no guanine was deposited even after standing several days. The second portion was agitated in the warm with silver oxide, filtered from silver-adenine, made alkaline with caustic soda and filtered from silver oxide. On heating the solution with Fehling's fluid, red cuprous oxide was promptly precipitated.

Elementary analysis of the crystalline nucleotide gave the following results:

- I. 0.3698 gave 0.2494. $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$.
- II. 0.2382 gave 0.1593. $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$.
- III. 0.2429 required 12.76 cc. of standard sulphuric acid.
(1 cc. = 0.00364 N).
- IV. 0.3969 required 20.85 cc. of standard sulphuric acid.
- V. 0.2750 gave 0.3293 CO_2 and 0.1114 H_2O .
- VI. 0.2808 gave 0.3366 CO_2 and 0.1072 H_2O .

	THEORETICAL FOR $\text{C}_{10}\text{H}_{14}\text{N}_4\text{PO}_7 \cdot$ H_2O	FOUND					
		I	II	III	IV	V	VI
P.....	8.49	8.54	8.44				
N.....	19.18			19.12	19.13		
G.....	32.88					32.66	32.69
H.....	4.38					4.50	4.24

The nucleotide is a dibasic acid. 0.1386 gram were suspended in hot water and titrated with a standard caustic soda solution, using litmus. 2.73 cc. of caustic soda were required (1 cc. of NaOH = 1.15 cc. of H_2SO_4 ; 1 cc. of H_2SO_4 = 0.00364 N)

Caustic soda required.....	per cent 23.06°
Theoretical for 2NaOH	22.22°

Experiments with the brucine salt also show that the nucleotide is dibasic.

The brucine salt of adenine nucleotide. The nucleotide unites with brucine to form a crystalline brucine salt of the composition

$C_{10}H_{14}N_3PO_7(C_{23}H_{26}N_2O_4)_2 \cdot 7H_2O$ which melts at 173 to 174°. It loses its water of crystallization slowly in a vacuum over sulphuric acid but rapidly at 105 to 110°. On exposure to air the dehydrated brucine salt takes up its water of crystallization sharply.

Four-tenths of a gram of crystalline nucleotide were suspended in hot water and neutralized to litmus with crystalline brucine. The nucleotide passed into solution before neutralization had been reached and before any brucine salt was precipitated, but as the solution cooled the finely crystalline brucine salt was deposited in bulk.

	grams
Nucleotide used.....	0.400
Brucine required.....	1.010
Theoretical amount.....	1.021

Specimens of the brucine salt were recrystallized in various ways; some from water, others from 35 per cent alcohol, and still others were recovered by the evaporation of aqueous or alcoholic mother liquors. All of these specimens behaved alike when heated in a capillary tube. The substance became contracted at 172°, showed signs of melting at 173° and at 174° had become a viscous brown liquid.

Brucine salt to the amount of 0.5306 gram was suspended in 30 parts of hot water and brought into solution by the addition of ammonia to alkaline reaction. After cooling (finally in the ice chest) the crystals of brucine were filtered off, washed, allowed to dry in the air and weighed (0.3766 gram). The ammoniacal filtrate was extracted with chloroform and a small additional amount of brucine thus obtained (0.0112 gram).

	per cen
Total brucine.....	73.1
Theoretical.....	73.9

The amino-nitrogen of adenine nucleotide. When treated at 20° with sodium nitrite and acetic acid in a nitrometer for the conventional three minutes, the nucleotide gives off an amount of gas that corresponds to only a small fraction of one atom of nitrogen. But in two hours the gas formation is fairly constant

and corresponds closely to one nitrogen atom. As this same conduct was exhibited by adenine sulphate, the adenine group cannot be united in the nucleotide by its amino-nitrogen atom (position 6 of the purine ring).

The data will appear in a publication by D. W. Wilson who has included adenine nucleotide in an examination of purine and pyrimidine derivatives which he is making.

Hydrolysis of the nucleotide with dilute sulphuric acid. This pure nucleotide is excellent material for an exact study of the rate at which phosphoric acid is set free from a purine nucleotide by hydrolysis with mineral acid. For this purpose weighed portions of nucleotide were heated with 5 per cent sulphuric acid for various periods, as formerly described for guanine nucleotide.³ At the end of each period a quantitative estimation was made both of free phosphoric acid and free adenine. The general results were as follows:

1. The phosphoric acid is set free in accordance with the rule previously set forth for purine nucleotides.

2. Both phosphoric acid and adenine are finally set free in theoretical amount.

3. The adenine is set free much more rapidly than the phosphoric acid. (At the end of fifteen minutes, 90 per cent of the one and 45 per cent of the other; or, the one requires for completion about thirty minutes, the other about 2 hours). As explained, this proves that the adenine group cannot be the central group of the nucleotide.

The data are reserved for another paper in which they may be compared with other similar data and their meaning treated in detail.

³ Jones and Read, Journ. Biol. Chem., xxxi, 337.

DRUG ACTION AS MODIFIED BY DISEASE TOXINS

I. OUABAIN VS. DIPHTHERIA TOXIN

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The object of this inquiry was to ascertain if the fairly-definite action of ouabain was modified to any appreciable extent by diphtheria toxin. Tests were first made on frogs, then on dogs. To the frogs the toxin was given in doses of 0.5 to 1 mil. administered by lymph sac. To the dogs, a uniform amount of 1 mil. was given hypodermically. No definite minimum lethal dose was determined for frogs; but 1 mil. of the toxin was found to cause death of a 5 kgm. dog in forty-eight hours.

Frogs were treated with the toxin forty-eight hours before the ouabain administration, while dogs were given the toxin but twenty-four hours ahead of the secondary experiment. Frogs were kept in uniform environment, each frog receiving the toxin being checked by another frog of the same approximate weight kept in the same cage and later tested with ouabain at the same time.

Four of the dogs used, 4, 8, 9, and 10, were from one litter, all males, and varied but little in weight. Dogs were much more satisfactory to use, of course, than frogs. The general unsatisfactoriness of frogs for this purpose, except as general indications, is shown by table 3. It was found necessary, as advised by Sollmann, to exclude from consideration all frog results departing widely from a fair mean. Frogs were anaesthetized by injecting three or four minims of 20 per cent urethane into the brain, using a hypodermic needle as a pithing needle. This method gives immediate relaxation; with subsequent muscle activity but rarely, and no hemorrhage; many checks indicated no definite cardiac action. Ouabain, in fresh solution, was ad-

ministered by the lymph sac, through the sinus, or by the drop method (3 mils. of a 0.01 per cent solution in fifteen minutes). This first method is least definite, because of uncertainty of absorption; the second (using a burette and a very fine hypodermic needle, and taking about five minutes to introduce 1 mil) gave fair results, though of course the drug was speedily diluted; the third method gave more nearly uniform graphs. A variation of the second method, possible only in the larger frogs, was intravenous administration of the ouabain through the abdominal vein.

Dogs were anaesthetized with chloretone, hydro-alcoholic solution, injected into the peritoneal cavity, 166 mg. per kilo dog. Operative anaesthesia was obtained in from eight to fifteen minutes.

With dogs, the ouabain in fresh warm ringer solution, was given by the femoral vein, a 0.01 per cent solution flowing into the vein at the rate of 0.75 mil. per minute.

The ouabain used was Merck's crystalline. The diphtheria toxin was furnished, standardized to guinea-pigs, by the H. K. Mulford Company, from their laboratories at Glenolden.

Reference to table 1 shows that all of the toxin frogs showed heart block, the average onset of which (omitting 19x and 19y) was about forty-one minutes. Only 40 per cent of the control frogs showed heart block, with an average onset of forty-four minutes. (The average weight of the toxin frogs was 55 grams of the control frogs, 53 grams.) The average dose of the toxin frogs (omitting 19x and 19y, and assuming that 43j and 44k would not utilize more than 25 per cent of the wash) was 0.0017 mgm. per gram; that of the control frogs was about double, 0.0033 mgm. per gram. The average time to ventricular arrest in the toxin frog was 61 minutes (including the probably aberrant 15b, but not including the very sensitive 19x and 19y); whereas in the smaller control frog, with double dose, the average time to ventricular arrest was 94 minutes in the three where arrest was observed. (The mark — before time given means "not in.")

With the dogs the average weight of the controls was 7.0 k; of the intoxicates, 8.2 k. The average dose to the controls was

0.215 mgm. per kilogram; to the intoxicates, 0.164 mgm. per kilogram. With the controls, the average time to the appearance of the first symptoms (arrhythmia) was forty-nine minutes; with the intoxicate twenty-nine minutes. With the controls, the

TABLE 1

NUMBER	WEIGHT	SEX	DIPHTHERIA TOXIN	AVENUE OF AD- MINISTRATION	OUABAIN	AVENUE OF OUABAIN	TIME OF FIRST IRRREGULARITY	TIME TO FIRST HEART BLOCK	TIME TO VENTRIC- ULAR ARREST
	grams		mils.		mgm. per gram				
11a	40	m			0.0062	l. s.	15	47	-3 hrs.
15a	55	f			0.0007	l. s.	35	50	2 hr. 15 m.
16a	45	f			0.0009	l. s. t.	24	35	-2 hrs.
18a	70	m			0.0017	l. s. t.	26	45	-1 hr. 30 m.
25a	60	m			0.0017	l. s. t.	40	None	-1 hr. 35 m.
26a	55	f			0.0015	l. s. t.	4	None	-1 hr. 20 m.
27a	55	f			0.001	l. s. t.	27	None	-1 hr. 20 m.
29	47	m			0.0022	l. s. t.	None	None	-1 hr. 30 m.
32	54	m			0.0095	l. s. t.	15	None	1 hr. 10 m.
34	54	m			0.0092	l. s. t.	38	None	1 hr. 16 m.
11b	42	f	1.0	l. s. t.	0.0061	l. s. t.	15	60	1 hr. 30 m.
15b	55	f	1.0	l. s. t.	0.0007	l. s. t.	None	1 hr. 25 m.	2 hrs. 10 m.
16b	45	f	0.5	l. s. t.	0.0009	l. s. t.	20	28	1 hr. 50 m.
18b	72	m	1.0	l. s. t.	0.0014	l. s. t.	12	21	50 m.
25b	60	m	0.75	l. s. t.	0.0017	l. s. t.	40	45	1 hr.
26b	55	m	0.75	l. s. t.	0.0015	l. s. t.	5	15	25 m.
27b	60	f	0.75	l. s. t.	0.001	l. s. t.	30	55	62 m.
20x	80	f	1.0	l. s. t.	0.0009	l. s. t.	15	27	63 m.
20y	65	f	1.0	l. s. t.	0.0011	l. s. t.	30	33	68 m.
19x	44	m	0.75	l. s. t.	0.0013	l. s. t.	6	7	30 m.
19y	45	m	0.5	l. s. t.	0.0013	l. s. t.	8	9	48 m.
42g	47	f	1.0	l. s. t.	0.0011	Sinus	21	36	50 m.
43j	35	m	1.0	a. l. s.	0.0085	Drop	10	32	40 m.
44k	44	m	1.0	l. s. t.	0.0066	Drop	8	52	68 m.

average time to first heart block was eighty minutes; with the intoxicates, fifty-three minutes. Arrest of the ventricles did not occur in two hours time with the controls, nor with two of the intoxicates; the average time of arrest with the other intoxicates was eighty-five minutes.

TABLE 2

NUMBER	WEIGHT	SEX	DIPHTHERIA TOXIN	AVENUE OF AD- MINISTRATION	MGM. X KGM.	AVENUE OF OUABAIN	TIME TO FIRST IRREGULARITY	TIME TO FIRST HEART BLOCK	TIME TO VENTRIC- ULAR ARREST
	kgm.		mils.						
1	5.8	f	1	Sub-q	0.116	Vein	18	68	1 hr. 38 m.
3	9.0	f	1	Sub-q	0.191	Vein	42	55	58 m.
4	6.5	m	1	Sub-q	0.230	Vein	19	39	1 hr. 24 m.
5	9.5	m	1	Sub-q	0.155	Vein	48	63	1 hr. 32 m.
6	10.0	f	1	Sub-q	0.150	Vein	15	35	-2 hrs.
8	6.0	m	1	Sub-q	0.150	Vein	41	62	1 hr. 34 m.
10	8.5	m	1	Sub-q	0.154	Vein	17	47	-2 hrs.
2	6.6	f	Control		0.191	Vein	37	74	-2 hrs.
9	7.5	m	Control		0.240	Vein	62	87	-2 hrs.

TABLE 3

NUMBER	WEIGHT	SEX	DIPHTHERIA TOXIN	AVENUE OF AD- MINISTRATION	OUABAIN	AVENUE OF OUABAIN	TIME TO FIRST IRREGULARITY	TIME TO FIRST HEART BLOCK	TIME TO VENTRIC- ULAR ARREST
	grams		mils.		mgm. per gram				
40	40	f			0.0062	Sinus	1 hr. 13 m.	2 hrs.	-2 hrs. 30 m.
43	45	f			0.001	Sinus		1 m.	4 m.
41	42	f	1	l. s. t.	0.0012	Sinus		5 m.	7 m.

CONCLUSION

These few experiments seem to indicate that the heart is definitely rendered more susceptible to ouabain by the diphtheria toxin.

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THE ACTION OF VIBURNUM PRUNIFOLIUM

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A review of the old clinical literature regarding the drugs efficient in the prevention of threatened abortion would lead the reader to believe that in the preparations of viburnum prunifolium there is available an unfailing remedy, one which quiets the uterus if that organ is stimulated to an abnormal degree of activity at an inopportune moment. The study of the action of certain proprietary remedies, one of the active principles of which was supposed to be derived from viburnum prunifolium, led us to test the action of the latter drug also. The results are far from conclusive as the table included will show, but the conclusions are warranted that none of the preparations of the drug which we employed markedly affect uterine movements, in either a positive or negative direction. We believe that the assertion is borne out by our experiments that as compared with pilocarpine and pituiturin, preparations of viburnum prunifolium must be considered an indifferent drug, with no specific action on the uterus. While our experiments are not very numerous, the results were so unpromising, that we did not consider the further sacrifice of animals necessary in continuing the study advisable.

METHOD

The investigation was carried out on various laboratory animals and both uterine strips and the uterus in situ were used. The uterine strips were taken from the horn and body of the uterus respectively and varied in length from $\frac{1}{4}$ to $\frac{3}{4}$ of an inch. These longitudinal strips were placed in 400 cc. of Tyrode's so-

lution at 36 to 37°C. Oxygen was passed through the solution throughout the test. One end was attached to a hook fixed under the solution while the other end was fastened by means of a silk thread to a writing lever. Many such strips were employed but only one is reported in this paper.

Fifty and seventy per cent alcoholic extracts were made by the maceration of finely ground *viburnum prunifolium* bark with alcohol. The decoction was prepared by boiling 10 grams of the finely ground bark in 200 cc. of water for twenty minutes.

The animals in which the uterus was retained in situ were first anaesthetized with ether, trephined through the parieto-temporal region, and the brain stem was severed by means of a knife handle. The hole in the skull was then plugged up with cotton and the skin flaps were stitched together. An abdominal incision about 3 inches long was made through the linea alba and the uterus was exposed. A fixed hook was placed in the wall of the ventral portion of the uterus while a stitch was taken through the wall of the dorsal portion and attached to the writing lever. The distance between the two hooks varied from $\frac{1}{2}$ to 1 inch. The animal was then submerged in a tank of 0.9 per cent salt solution to which 1 gram of sodium bicarbonate per liter was added. The temperature of the solution was maintained at 37 to 39°C. by means of an electric stove. About an hour was allowed to elapse between the high section and the injection of the drug.

Since it is evident that stimulation of the uterus may constitute a stimulation in the rate of the rhythmical contraction, or in the amplitude, of the individual contraction or in the tone of the whole uterus, the experiments were conducted with the idea in mind of securing all the data possible on these different functions. In the work on the uterus the writers find that the greatest care must be exercised to eliminate modifications of uterine movements due to causes aside from the action of the drugs studied. Alcohol injected intravenously either by its own action chemically, or reflexly by stimulation of nerves by the injection affects the nature of uterine action. This is borne out by the following experiment (see tracing 1).



FIG. 1. Dog, NONPREGNANT

(1) Normal. (2) after 5 cc., 70 per cent alcohol intravenously 27 minutes. (3) After 5 cc., 70 per cent alcohol intravenously 60 minutes. (4) After 5 cc. 70 per cent alcohol intravenously 80 minutes. (5) After viburnum prunifolium intravenously (5 cc. 70 per cent alcohol extract) 20 minutes. (6) After viburnum prunifolium 2 hours. (7) after viburnum prunifolium 2½ hours. This graph shows: (a) the depressing effect upon the uterus of the intravenous injection of alcohol, (b) a stimulating effect of viburnum prunifolium, reaching a maximum 20 minutes after the intravenous injection of the drug, and then gradually decreasing for 2 hours.

Dog, non-pregnant

- 7.30 High section.
1. 8.25 Normal uterine contraction.
 2. 8.52 After 5 cc. 70 per cent alcohol, 27 minutes previously.
 3. 9.25 After 5 cc. 70 per cent alcohol, 60 minutes previously.
 4. 9.45 After 5 cc. 70 per cent alcohol, 80 minutes previously.
 5. 10.36 After 2 cc. 70 per cent alcoholic extract viburnum prunifolium 20 minutes previously.
 6. 12.16 After 2 hours. After viburnum prunifolium.
 7. 12.45 After 2½ hours. After viburnum prunifolium.

In this experiment as can be seen from the tracing it is evident that the injection of alcohol inhibited the uterine contraction. This inhibition passed off and was followed by stimulation and a depression after viburnum prunifolium.

The effect of viburnum prunifolium upon the uterus in situ is shown best by the use of graphs.

Figure 2 shows a long time (two hours) experiment upon a pregnant rabbit, and shows the effect of a single intravenous dose, followed by primary inhibition, due either to the drug or to the 70 per cent alcohol in the extract, and secondary increase, but not markedly above the normal.

Figure 3 shows another long time experiment, showing the effect of alcohol, repeated injection of alcoholic extract of viburnum prunifolium, and of ergot. In this case the viburnum prunifolium is clearly stimulating in effect, but not nearly so stimulating as the ergot used at the end of the experiment.

Figure 4 shows the effect of viburnum prunifolium injected into the bowel.

The fact that a slight change in the frequency and amplitude of the contractions may occur immediately on inserting the needle into the bowel suggests that the slight increase in the strength of the contractions is brought about reflexly. Manipulation of the animal in the slightest manner interferes with, and modifies the character of the uterine contraction. The fact has been demonstrated time and again throughout the progress of this investigation. The change in the rate and size of the contractions which occur after injecting viburnum prunifolium as compared

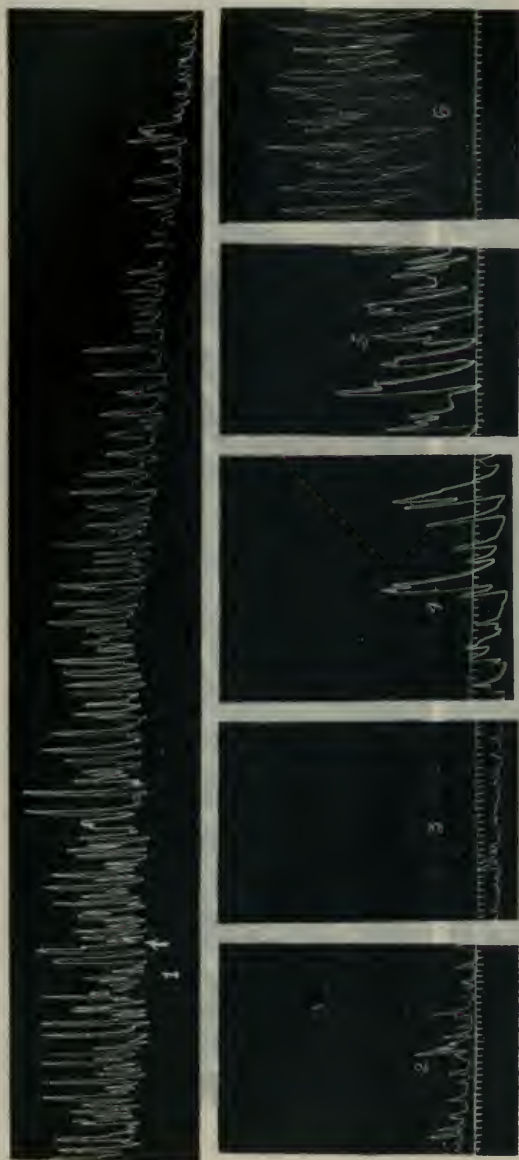


FIG. 2. RABBIT, PREGNANT

(1) 9.10 Normal, at \uparrow 2 cc. 70 per cent alcoholic extract of viburnum prunifolium was injected, intravenously. Note depression beginning almost immediately. (2) 9.40. (3) 10.05. (4) 10.25. (5) 10.45. (6) 11.00.



FIG. 3. DOG, NONPREGNANT

(1) 5.15 Normal followed by the injection at \uparrow of 5 cc. 30 per cent alcohol. Note slight stimulation. (2) 5.25 Injection intravenously of 5 cc. 30 per cent alcoholic extract of *viburnum prunifolium*. (3) 5.55 5 cc. as in (2). (4) 6.05 5 cc. as in (2). (5) 6.15 10 cc. as in (2). (6) 6.40 1 cc. ergot. This graph shows: (a) the stimulating action of alcohol on the uterus, (b) the stimulating effect (mild) of *viburnum prunifolium*, (c) the stimulating effect (strong) of ergot.



FIG. 4. Dog, NONPREGNANT

(1) 9.00 Normal. At \uparrow 8 cc. 50 per cent alcoholic extract of *viburnum prunifolium* injected into bowel. (2) 9.10. (3) 10.10. Note decreased tone of the uterus between (1) and (2) with a slight increase in the succeeding hour.

TABLE 1
Detailed effects of *viburnum prunifolium* on the uterus

EXPERIMENT	ANIMAL	STATE	DRUG			NORMAL			EARLY EFFECT			LATE EFFECT		
			Amount	Route	Form	Rate per hour	Amplitude	Tone	Time after	Rate per hour	Amplitude	Time after	Rate per hour	Tone
1	Rabbit	Pregnant	25 cc.	Bowel	Decoction	36	90	30	5	48	94	20	36	65
2	Rabbit	Nonpregnant	25	Bowel	Decoction	36	65	40	5	60	95	55	60	40
3	Dog	Nonpregnant	10	Bowel	Decoction	276	4	30	5	288	2	Died	60	30
4	Dog	Nonpregnant	2	Bowel	50 per cent alcohol extract	144	3	10	5	168	3	20	204	3
5	Dog	Pregnant	4	Bowel	50 per cent alcohol extract	276	4	10	5	216	4	50	276	12
6	Dog	Nonpregnant	8	Bowel	As in 3	168	10	38	5	168	10	70	144	18
7	Dog	Nonpregnant	50	Bowel	Decoction	30	30	30	5	12	19	40	48	5
8	Rabbit	Nonpregnant	5	Bowel	Alcohol extract	84	6	22	5	60	7	150	60	8
9	Cat	Nonpregnant	10	Bowel	Decoction	36	40	8	5	24	42	56	30	34
10	Dog	Nonpregnant	75	Bowel	Decoction	42	35	6	5	48	25	50	108	19
11	Rabbit	Nonpregnant	20	Intravenous	Decoction	204	8	21	5	240	12	60	132	18
12	Dog	Nonpregnant	50	Intravenous	Decoction	18	3	19	5	18	5	20	24	5
13	Dog	Nonpregnant	2	Intravenous	70 per cent alcohol extract	228	3	20	5	288	5	Died	24	30
14	Cat	Nonpregnant	5	Intravenous	70 per cent alcohol extract	156	18	5	5	180	12	117	216	48
15	Dog	Nonpregnant	5	Intravenous	70 per cent alcohol extract	24	8	5	5	120	4	60	192	9
16	Dog	Nonpregnant	8	Intravenous	70 per cent alcohol extract	96	32	55	5	96	26	18	72	57
17	Guinea-pig	Nonpregnant	4	Intravenous	70 per cent alcohol extract	108	20	25	5	84	20	60	60	4
			2	Subcutaneous	70 per cent alcohol extract	36	7	5	5	48	7	20	24	8
				Strip		390	3	30	3	486	3	80	312	2

with the stimulation and contractions which occur following the injection of pituitary extract is negligible.

The results secured from nineteen experiments are recorded in table 1.

The results of table 1 are summarized in table 2.

As can be seen from these tables the rate, amplitude and tone of the uterine mechanism in the normal animal varies between exceedingly wide limits: there is in fact no level which could be picked out as being the normal; each uterus is a law for itself. Only two experiments of our series listed were performed on pregnant animals, but these experiments were in no way different from the nonpregnant ones. So far as rate is concerned, five

TABLE 2

Summary of the effect of viburnum prunifolium on movements of the uterus with special reference to the rate and amplitude of the contraction, and the tone of the organ

	EARLY (5 MINUTES AFTER THE APPLICATION OF THE DRUG)			LATE (18 TO 150 MINUTES AFTER THE APPLICATION OF THE DRUG)		
	Rate	Amplitude	Tone	Rate	Amplitude	Tone
Increased.....	11 (57.8%)	7 (36.8%)	11 (57.8%)	6 (35.3%)	8 (47.0%)	10 (58.5%)
Decreased.....	5 (26.3%)	6 (31.4%)	4 (21.4%)	8 (47.0%)	8 (47.0%)	5 (29.4%)
No change.....	3 (15.7%)	6 (31.4%)	4 (21.4%)	3 (17.6%)	1 (5.8%)	2 (11.6%)
Total.....	19	19	19	17	17	17

minutes after the drug was applied the contractions were increased in 57.8 per cent, decreased in 26.3 per cent, and unchanged in 15.7 per cent of cases; a later observation showed an increase in 35.3 per cent, a decrease in 47 per cent, and no change in 17.6 per cent of cases. It is safely concluded therefore that the drug is by no means an effective uterine sedative, at least so far as the number of contractions are concerned. Turning now to *amplitude*: Five minutes after the application of the drug, the amplitude was increased in 36.8 per cent, decreased in 31.4 per cent, and unchanged in 31.4 per cent of cases; after the longer period of action of the drug the amplitude was increased in 47 per cent, decreased in 47 per cent, and unchanged in 5.8 per cent of cases. The conclusion is warranted that viburnum prunifolium is not an effective depressant of the uterus as far as each individual

contraction is concerned. Considering now the *tone* of the uterus as a whole: In five minutes there is increased tone in 57.8 per cent, decreased tone in 21.4 per cent, and no change in 21.4 per cent of cases; in the longer period there was increased tone in 58.8 per cent, decreased tone in 29.4 per cent, and no change in 11.6 per cent of cases. It is therefore evident that *viburnum prunifolium* has a tendency to an increase in tone of the uterus, and as a result might be classed as a variety of uterine stimulant rather than depressant, for as is well known from the study of smooth muscle in other places an increase in the tone of the muscle increases the tendency to rhythmical contraction.

CONCLUSIONS

The results of this investigation indicate that the effect produced on the uterus by alcoholic extracts and decoctions of *viburnum prunifolium* bark are of little consequence in modifying the nature of the uterine activity. No uniform pharmacologic effect can be ascribed to the drug, for while a stimulation may seem evident at one time, a similar dose under the same conditions produces an apparent inhibition or no perceptible change whatever. As compared with drugs known to have a specific action on the uterine contractions such as pilocarpine and pituitary extracts in case of the pregnant uterus, the effect is negligible. The change in the contractions of the uterus which sometimes occur on the addition of an extract of the *viburnum prunifolium* bark are so slight that the changes may be explained as having been produced reflexly through manipulation of the animal during injection or by the alcohol which holds the drug in solution. It is quite evident that the uterus of animals rendered unconscious by high section respond to the intravenous injection of alcohol and a temporary inhibition or stimulation of the uterus is produced. As far as can be ascertained from the use of laboratory animals, no specific action on the uterus can be ascribed to preparations of the bark of *viburnum prunifolium*.

We wish to express our indebtedness to Prof. A. P. Mathews for his coöperation and encouragement during the course of the work.

ON OPTICAL ISOMERS

V. THE TROPEINES¹

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ATROPINE AND *d*-HYOSCYAMINE

In 1904 I stated that *l*-hyoscyamine acted in the periphery about twice as strongly as *dl*-hyoscyamine (atropine) and 12 to 15 times as strongly as *d*-hyoscyamine (1). The *d*-hyoscyamine used was part of the original preparation of Gadamer and may not have been absolutely free from the *laevo*-rotary isomer. Laidlaw (2) using a preparation made by Barrowcliff and Tutin, found *l*-hyoscyamine about 100 times as powerful as the *d* base on the iris and at least 25 times as powerful on the cardiac vagus. I have now tested a preparation of *d*-hyoscyamine prepared by Tutin, comparing it with pure atropine.

The method was that used in some of my former experiments and measured the degree in which these alkaloids are capable of antagonising pilocarpine.

A small smooth-haired terrier bitch, "Vic," weighing 5.5 kgm. in the beginning of the series and 7 kgm. at the end, had a permanent fistula made in her right submaxillary duct, and a month afterwards the series of experiments was begun. She was taken out for a run in the morning an hour before the observations began and received no food until an hour after they were ended.

¹ Previous papers of this series have appeared in the *Journal of Physiology* as follows:

1. Atropine and the hyoscyamines, xxx, 176, 1904.
2. Hyoscines, xxxii, 501, 1905.
3. Adrenaline, xxxvii, 130, 1908.
4. Adrenaline, xxxviii, 259, 1908-1909.

An interval of at least twenty-four hours and often of forty-eight hours or more intervened between two observations. The animal lay on the table and after the first few days viewed the proceedings with indifference, generally falling asleep except when nausea or vomiting was induced.

The opening of the fistula was carefully wiped dry and a subcutaneous injection of the tropeine under examination was made generally in the back. As a general rule the tropeine was dissolved freshly each day, and this was invariably the case when the less active bodies to be discussed later were used. The pilocarpine solution was a stock one containing 5 mgm. per cubic centimeter.

The saliva secreted each five minutes was collected on a small weighed pledget of cotton wool and the amount was ascertained by re-weighing. Ten minutes after the tropeine injection, 5 mgm. of pilocarpine nitrate was injected subcutaneously and the secretion was collected for each five minutes for the next forty minutes. The results were then put in the form of a graph which was compared with that obtained from an experiment in which the other tropeine had been used in the same way. The experiment was then repeated with different doses of each drug until the graphs approached each other sufficiently closely. In the figures the graphs are either those of individual experiments which followed closely upon each other, or in other cases are drawn from a series of observations made at longer intervals with the same dose. The drugs were used in the form of salts, but in each case the solution was made up to contain a definite amount of base. The result ordinarily reached was that some quantity x of the hyoscyamine was weaker than y of atropine, while $x + a$ of hyoscyamine was stronger than y of atropine. Several determinations were made for each alkaloid and the results were in accord. The method does not, of course, admit of absolute accuracy, but that it gives fairly constant results is shown by the similarity of the graphs taken under the same conditions at intervals of several months. In figure 1 two graphs of the secretion under pilocarpine after 0.1 mgm. atropine are given. Several other experiments under the same dose gave similar results,

but are omitted to avoid confusion of the graphs. The rate of the pulse was taken in the thorax or in the femoral artery every five minutes throughout the observation and any symptoms of nausea or vomiting were noted.

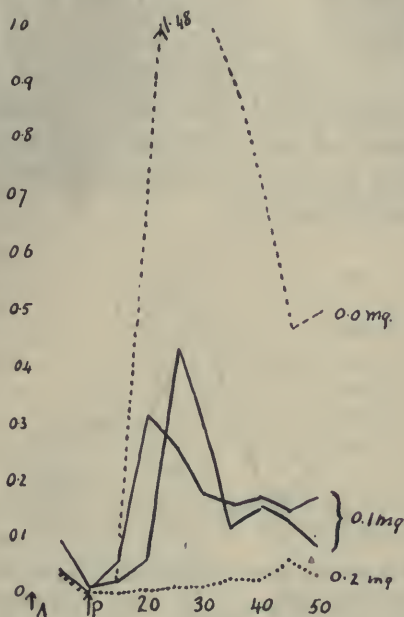


FIG. 1. The amounts of secretion are shown along the vertical, the time along the horizontal lines. At A injection of atropine, at P injection of 5 mgm pilocarpine. The broken line, - - - indicates the secretion under pilocarpine alone. The two unbroken lines that under pilocarpine preceded by an injection of 0.1 mgm. atropine (two experiments at an interval of some months). The dotted line, . . . the secretion under pilocarpine preceded by 0.2 mgm. atropine. Under pilocarpine alone, the secretion reached 1.48 cc. in five minutes, but the curve has not been drawn to this point in order to save space.

The results (see fig. 2) show that 2 mgm. *d*-hyoscyamine is almost equivalent to 0.1 mgm. atropine, while 3 mgm. is much more powerful. This is in accord with several other measurements and indicates that on the terminations of the chorda tympani atropine acts about 20 times as strongly as *d*-hyoscyamine; *l*-hyoscyamine would therefore have an action 40 times as great as *d*-hyoscyamine.

My former results with Gadamer's *d*-hyoscyamine thus appear to have given it a higher value than it merits, probably because it was not completely free from the *l*-form. On the other hand Laidlaw's observations on the mydriatic effects of the two isomeric hyoscyamines give a ratio of 1:100 in favor of the laevo-rotary and this puts the action of the *d*- at too low a figure; his ratio of 1:25 for the cardiac vagus corresponds better with mine for the chorda tympani and there can be no question¹ that the action of the pulse and secretions can be measured more accurately than that on the iris if the drug is merely dropped on the conjunctiva in the last method.

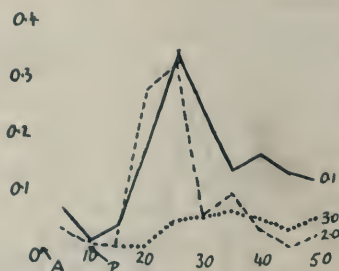


FIG. 2. Graphs of secretion under pilocarpine 5 mgm. (at *P*) after the injection at *A* of 0.1 mgm. atropine (—), 2.0 mgm. *d*-hyoscyamine (---), and 3 mgm. *d*-hyoscyamine (....) respectively.

The normal pulse rate of this dog varied from 90 to 130 on different days and at different stages of the same experiment, generally falling throughout the observations when no drug was given or when Ringer's solution was injected. When a large dose of atropine was injected, it rose to 210–220. I found that 0.4 mgm. atropine was sufficient to cause this maximal acceleration for at least twenty minutes, 0.2 mgm. gave a rate of 180 to 200, and 0.1 mgm. one of 150 to 160. Of *d*-hyoscyamine 10 mgm. increased the pulse to 220, 3 mgm. gave 160, while 1 mgm. caused no acceleration. When the pulse rose to 160 it became perfectly regular, the respiratory arrhythmia disappearing so far as could be made out by the finger. The difference between the effects of atropine and *d*-hyoscyamine on the cardiac inhibition

is thus of the same order as that on the salivary secretion, but I have not followed the changes in the pulse rate so closely.

HOMATROPINES

Racemic homatropine was obtained from Burroughs Wellcome and Company in the form of the hydrobromide. It was formed from racemic mandelic acid and had a melting point of 210° (uncorrected), so that it was chemically pure. *d*-homatropine was kindly prepared for me by Dr. Pyman, Director of the Wellcome Chemical Research Laboratories and his colleague,

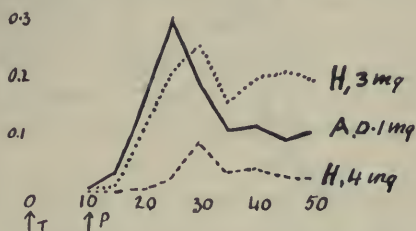


FIG. 3. Graphs of secretion under pilocarpine 5 mgm. (at *P*) after the injection (at *T*) of 0.1 mgm. atropine (—) 3, mgm. *dl*-homatropine (. . .), and 4 mgm. *dl*-homatropine (- - -) respectively.

Mr. H. King, who informed me that it is purer than the sample previously prepared by Jowett and Barrowcliff and examined by Dale (3), 3 mgm. of racemic homatropine (calculated as base) proved practically equal to 0.1 mgm. atropine, while 4 mgm. were distinctly stronger (fig. 3). The graphs are similar in character, showing that the destruction of these alkaloids in the tissues is of the same character. Atropine is thus about 30 times as effective as racemic homatropine. No exact values for atropine and homatropine have been available hitherto, but the ratio of 1:30 appears to agree fairly with clinical experience to judge from the British Pharmacopoeia, which orders lamellae of atropine to contain 1/5000 gr., while those of homatropine contain 1/100 gr. i.e., the relative activity is held to be 1:50.

d-homatropine was compared with *dl*-homatropine. Dale found *d*, *l* and *dl*-homatropine all equal in mydriatic power

(local application, Jowett and Pyman), but his method did not allow of such fine estimations as mine and in addition his *d*-homatropine was less pure. I find that 4 mgm. *d*-homatropine is less effective than 3 mgm. *dl*-homatropine but more effective

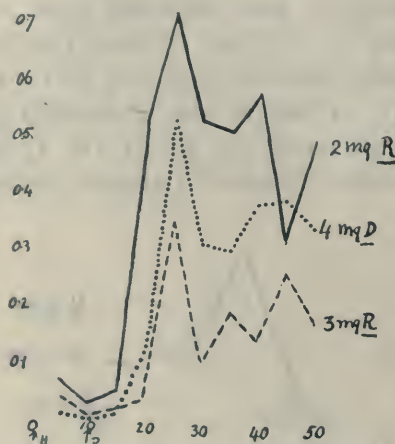


FIG. 4. Graphs of pilocarpine secretion after the injection of 2 mgm. *dl*-homatropine (—), 3 mgm. *dl*-homatropine (---), and 4 mgm. *d*-homatropine (....) respectively.

than 2 mgm. (fig. 4). Taking 4 mgm. *d* as equivalent to 2.5 mgm. *dl*, we get the equation:—

$$4d = 2.5 dl = 1.25 d + 1.25 l$$

and therefore

$$2.75d = 1.25 l$$

or neglecting fractions

$$2d = l$$

So that *l*-homatropine is approximately twice as effective as its *d* isomer.

DISCUSSION

The *l*-homatropine is twice as active as the *d* form, while *l*-hyoscyamine is 40 times as powerful as its *d* isomer. The influence of the change in sign thus differs widely in different tropanes, and this leads to a consideration of its underlying cause. Optical isomers differ in the direction in which they rotate the

plane of polarized light, in their crystalline form, in the fact that their compounds with other optically active substances differ in solubility, and lastly in their relation to living organisms. It does not seem promising to attempt to bring the first two qualities into relation with the effects on living tissues, and I have repeatedly suggested that these latter may be compared to the solubility variations of the compounds with optically active substances. Living matter is itself optically active, that is, contains asymmetric carbon atoms and the difference in the action of the two hyosecyamines in living organisms may plausibly be explained by their forming compounds with the molecules containing asymmetric carbon, these compounds differing in solubility or other physical characters. Now it is well known, that different pairs of isomers do not necessarily show the same degree of divergence when compounded with the same optically active substance. For example, the relative solubility of the camphor sulphonates of the two hyosecyamines is not the same as that of the two homatropine camphor sulphonates. In the same way the relative activity of the two hyosecyamines in the tissues need not necessarily be the same as that of the two homatropines. There can be no question that these tropeines act on the same substance or receptor, and this receptor must have asymmetric carbon atoms, which differ in the qualities of the compounds which they form with the alkaloidal isomers. The compounds formed with *l*-hyosecyamine may be very insoluble, that with *d*-hyosecyamine less insoluble; the compound with *l*-homatropine nearly as soluble as that with *d*-hyosecyamine, and while that with *d*-homatropine would be the most soluble of all; the difference in the solubility of the two homatropine compounds would be much smaller than that between the two hyosecyamines. The tropeines without asymmetric carbon can also react with the same receptor just as they form compounds with camphorsulphonic acid, but so far no such tropeine is known which forms so "insoluble" compounds with the receptor. At first sight this may seem to suggest that the reaction of these bases with the receptor is a purely chemical one and that their pharmacological action depends on this, which would run counter

to the tendency of pharmacological thought at present, and would be in direct opposition to previous work (5) in which I showed that the activity of atropine depends on the law of mass action and not on that of chemical affinity; but this would be an erroneous deduction. It is true that I assume some chemical reaction to occur between the receptor and the alkaloid, but this follows whether the *l* or the *d* form is present, just as in their reaction with *d* camphorsulphonic acid, which combines with either *d* or *l*-hyoseyamine indifferently. The difference in action lies not in the facility with which the chemical combination is formed, but in the physical characters of the resultant compound. In the case of camphorsulphonic acid these compounds differ in solubility and some similar difference in the compound of the living receptor and the alkaloid may explain the marked change in the pharmacological activity which accompanies the change in sign.

SOME OTHER TROPEINES

Since atropine was shown to be tropyl-tropeine, the effect of substituting other acids for tropic acid in the molecule has been investigated repeatedly. The first to take up this question was Buchheim (11), who was soon followed by Eckhard (12), to whom he supplied his preparations of benzoyltropine and belladonnine; neither of these was in a chemically pure state, for Ladenburg (13) states that Buchheim's benzoyltropine contained only a small proportion of the pure substance, and his method of isolating belladonnine is obviously not adapted to remove traces of atropine from the mixture from which he started. Ladenburg (14) formed a number of artificial tropeines and had their mydriatic properties tested by Falek and others. Gottlieb (15) examined the action of several tropeines on the iris and heart. The most extensive work on this series is that of Jowett and Pyman (3), who give a list of no less than 40 tropeines; the activity as mydriatics of a few of these had been determined previously, and that of the others was tested by Marshall and Dale by instillation into the cat's eye. A later publication by Pyman (4) gives the mydriatic action of five further tropeines, making a total of 45 members of this series.

The object of these experiments has generally been to determine whether the tropeines are mydriatic or not, and little attempt was made to find out their relative activity except by Dale, who compared many of these by dropping two solutions equivalent in tropeine content into the eyes of a cat and observing the degree of dilatation of each pupil, and the rapidity of onset. In this way, the relative power of a large number of pairs could be rapidly ascertained; the standard taken was homatropine. No actual numerical values are given, and indeed it seems impossible to obtain them by this method, since the amount of the tropeine that reaches the iris must vary considerably.

The doctrine has received a wide currency that only tropeines of a certain chemical structure have the characteristic atropine action in the periphery; the acid must contain a benzene nucleus, and an alcoholic hydroxyl group in the side chain containing the carboxyl group. This generalization is often associated with Ladenburg's name, but is disclaimed by him (Jowett and Pyman). The earliest statement of the second qualification is found in Pictet's *Pflanzenalkaloide* (1891, p. 134). Both qualifications have been shown to be too absolute by Jowett and Pyman, who found that a pyridine nucleus could be substituted for benzene without the disappearance of the action, while benzoyltropeine is active yet contains no alcoholic hydroxyl. They therefore concluded that no generalization can be made at present as to the relation between mydriatic action and chemical constitution. Too often the attempt has been made to draw an arbitrary line above which all the tropeines are pharmacologically active, while below all are inactive. Different authors draw the line at different levels of activity, and thus a tropeine said to be inactive by one because it does not dilate the pupil in 1 per cent solution, is found active by another because it releases the frog's heart. Perhaps it is preferable to indicate stronger and weaker action, the latter shading off into inactivity, rather than to adopt such arbitrary divisions.

In view of the great difference in the power of the optical isomers, it appeared to me that the importance of the presence of the asymmetric carbon atom in the acid component of the

tropeines had not been recognized, and that the comparison of atropine and homatropine with simpler tropeines containing no asymmetric carbon might throw some light on the question. Dr. Pyman kindly sent me a number of these simpler tropeines for examination by the permanent fistula method, and I have also tested most of them, by instillation into the cat's eye.

Among the aliphatic compounds with tropeine, I have tested *d*-tartryl-tropeine prepared by Pyman. This was found devoid of mydriatic properties by Dale, and in my experiments the injection of 30 mgm. had no effect on the salivary secretion and did not modify in any way the action of pilocarpine. This result is in accord with the general view that aliphatic tropeines are devoid of the peripheral atropine action. Observations which run counter to this rule are those of Gottlieb (15), who found that lactyl-tropeine has some slight action on the iris, vagus and chorda, and of Marshall (16), who states that terebyl-tropeine is feebly mydriatic and has a weak action on the vagus; Dale did not find any mydriasis from terebyl-T by his comparative method. Methylparaconyl-T has an even feebler action on the vagus than terebyl-T (Marshall). Eight aliphatic tropeines are given by Pyman as having been examined by various observers:²

Acetyl-T, CH_3COOTNo mydriatic action

Glycolyl-T, CH_2OHCOOTNo mydriatic action

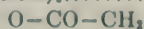
dl-Lactyl-T, $\text{CH}_3\text{CHOHCOOT}$

Slight mydriasis; weak vagus action (Gottlieb) (15)

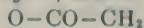
Succinyl-T ($\text{CH}_2\text{CO}_2\text{T}$)₂.....No mydriatic action

d-Tartryl-T (CHOHCOOT)₂.....No mydriatic action

Fumaroyl-T (CHCOOT)₂.....No mydriatic action



Methylparaconyl-T, $\text{CHMe}-\text{CH}-\text{COOT}$.Weak vagus action (Marshall) (16)



dl-Terebyl-T, $\text{C}(\text{Me})_2-\text{CH}-\text{COOT}$Slight weak vagus action (Marshall)

² In this list and in those following the tropeines with asymmetric carbon are in italics and the asymmetric carbon atom is indicated by black-face type. T indicates Tropine.

Of these, four have no asymmetric carbon and no claim for mydriatic action has been raised on their behalf. Of the four with asymmetric carbon, one, the tartryl-T, has been examined only in the dextrorotary form, which would not be expected to act strongly; the three others appear to have very slight action, but mydriasis is claimed for two of them and the specific action on the vagus antagonising pilocarpine for all three. It is clear that the action of the aliphatic tropeines is so small as to approach the vanishing point, but it is significant that those for which the specific atropine action has been claimed are all three *dl* compounds containing asymmetric carbon. The action is so slight, however, in any case, that it did not promise to repay further investigation.

A number of tropeines of the simpler aromatic acids have been examined.

Benzoyl-tropeine was found by Buchheim and Eckhard to possess the peripheral action of atropine, but the preparation used by them was shown to be impure. The pure tropeine prepared by Jowett and Pyman proved mydriatic but the order of activity was low.

The *o*-hydroxybenzoyl-otropeine (salicyl-tropeine) was stated by Ladenburg (Falek) to be inactive in the iris, but possesses a fairly high degree of activity (Jowett and Pyman).

The *m*-hydroxybenzoyl-ester was found weakly mydriatic by Ladenburg (Volkers) and is assigned some activity by Jowett and Pyman, while the para-isomer was found inactive by them. The phthaloyl and protocatechoyl-tropeines are stated to be not mydriatic (Jowett and Pyman). Phenylacetyl-T is mydriatic (Jowett and Pyman). I have examined the benzoyl, the three oxybenzoyl and phenylacetyl tropeines sent me by Dr. Pyman and the results are represented graphically in figures 5-9. In figure 5, the curve of secretion under pilocarpine after 20 mgm. benzoyl-tropeine is higher throughout than that under pilocarpine after 0.1 mgm. atropine, so that atropine has more than 200 times as much antagonistic power as benzoyl-tropeine. On the other hand, the curve of secretion after 30 mgm. of benzoyl-tropeine lies lower than that after 0.1 mgm. atropine in the first

thirty minutes, though afterwards it rises higher, and finally reaches the level of the later stages of pilocarpine injected without any antagonist. This indicates that 30 mgm. is rather more efficient as an antagonist at first, but loses its activity sooner than 0.1 mgm. atropine, through being oxidized or excreted. It may be taken that 30 mgm. benzoyl-T is more effective than 0.1 mgm. atropine as long as it remains intact. The efficiency of atropine is thus more than 200 times and less than 300 times that of benzoyl-tropeine.

Under benzoyl-tropeine 30 mgm. the pulse showed no acceleration in one experiment, while in another it rose to 150 per

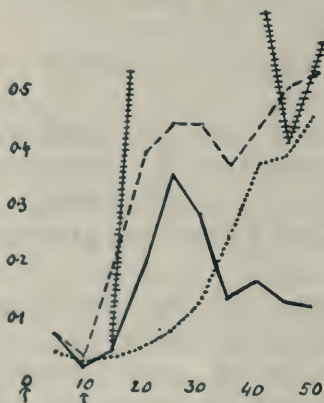


FIG. 5. Graphs of pilocarpine secretion after the injection of 0.1 mgm. atropine (—), 20 mgm. benzoyl tropein (---), and 30 mgm. benzoyltropein (....) respectively. The beginning and end of a pilocarpine secretion without any tropeine are shown (xxxx).

minute. The action on the vagus is thus very slight. No retching or vomiting was observed from pilocarpine after 30 mgm. benzoyl tropeine, but these were not prevented by 20 mgm.

The effect on the iris was tested by dropping four drops (0.12 cc.) of a solution on a cat's eye and comparing the degree of dilatation with that of a 1:50,000 solution of atropine ascertained some days previously. It was found that 1 per cent of benzoyl-tropeine had approximately the same action as 1:50,000 atropine. This method does not admit of such close approximation as the salivary experiments.

Salicyl-tropeine 30 mgm. was practically equivalent to 0.1 mgm. atropine in antagonistic action (fig. 6), and maintained its effect equally well. The ratio is thus about 1: 300.

The pulse was very little accelerated in one experiment under 30 mgm. of salicyl-tropeine, and in another some retching but no vomiting occurred. The cat's pupil was partly dilated by a 1 per cent solution, approximately to the same extent as by 1/50,000 atropine.

m-Oxybenzoyl-tropeine was equivalent to the ortho isomer at first but lost its effectiveness sooner, so that the saliva increased gradually in quantity. The pulse rose to 160 after 30 mgm. were injected. The pilocarpine retching and vomiting were not

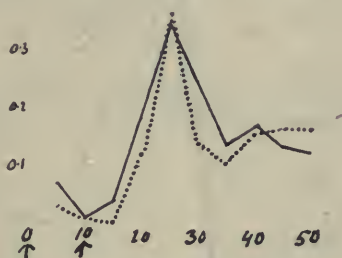


FIG. 6. Graphs of pilocarpine secretion after the injection of 0.1 mgm. atropine (—), and 30 mgm. salicyl-tropeine (....) respectively.

prevented by the previous injection of 30 mgm. *m*-oxybenzoyl-T. The cat's pupil was slowly dilated by a 1 per cent solution.

p-Oxybenzoyl-tropeine had no apparent action on the salivary secretion in doses of 30 mgm. (fig. 7). The pulse was not definitely accelerated and pilocarpine caused retching and vomiting. The cat's pupil showed a very slight but quite definite widening from 1 per cent solution after three hours; the dilatation is less than that from 1/50,000 atropine.

Phenylacetyl tropeine was not examined so completely as the others, since the quantity available was small; and while several observations gave it a value of the same order as benzoyl-T, I did not complete these at the time. Some months later when I had leisure to return to these experiments, I found that the dog had now developed a new reaction; the injection of phenyl-

acetyl-T was followed immediately by a profuse secretion of saliva, which slowly decreased during the succeeding periods but precluded further comparisons. Benzoyl-tropeine had now the same effect, while the injection of Ringer's solution caused no secretion. The salivation from the tropeines commenced within a minute of the injection and was obviously reflex in character. It stopped further experiments on this dog and I cannot feel the same confidence in the measurements under phenylacetyl-T as in those of the other tropeines. At the same time the fact

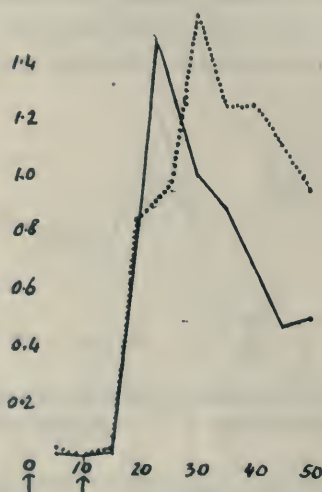


FIG. 7. Graphs of pilocarpine secretion without any previous tropeine injection (—), and after the injection of 30 mgm. *p*-oxybenzoyltropeine (....). The scale in this figure is only one-half of that in the others.

that this reflex secretion was not arrested by even 30 mgm. shows the weakness of its action.

The earlier observations with phenylacetyl-T showed that 20 mgm. were less effective than 0.1 mgm. atropine, and that 30 mgm. delayed the salivation from pilocarpine, but had not more effect in the end than 0.1 mgm. atropine (fig. 8). The ratio may thus be taken as 1:300.

On comparing the graphs from *dl*-homatropine and phenylacetyl-T (fig. 9), the former is found to be about ten times the

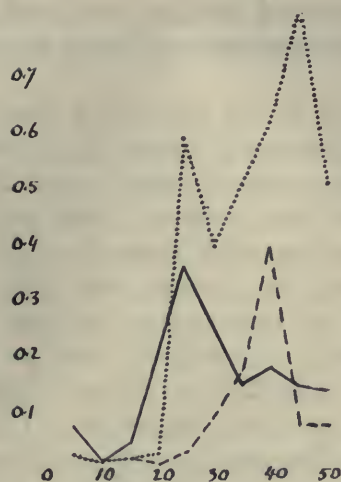


FIG. 8. Graphs of pilocarpine secretion after 0.1 mgm. atropine (—), 20 mgm. phenylacetyl-tropeine (....) and 30 mgm. phenylacetyl tropeine (---) respectively.

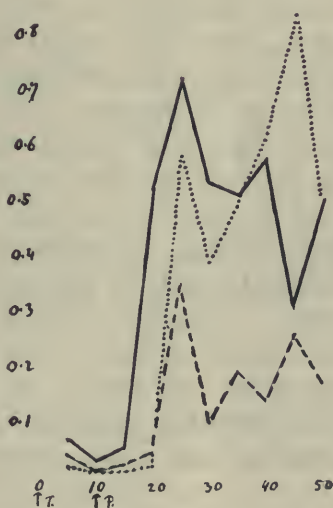


FIG. 9. Graphs of pilocarpine secretion after phenylacetyl 20 mgm. (....), *dl*-homatropine 2 mgm. (—), and *dl*-homatropine 3 mgm. (---) respectively.

stronger, and from this it may be calculated that *l*-homatropine is 14 times and *d*-homatropine 7 times as powerful as phenylacetyl-T.

Exact comparison of the activity of these weak tropeines is difficult, but the benzoyl-T seems on the whole slightly more efficient than *o*-oxybenzoyl-T, which again is superior to the *m* variety, and much the weakest is the *p*-oxybenzoyl-T. The inefficiency of *m*-oxybenzoyl-T seems due to its rapid elimination on destruction in the tissues and this may obtain in higher measure in the para-isomer; the last is so weak that no definite antagonism to pilocarpine could be made out when it was injected, but quite distinct though slight dilatation of the pupil was elicited by local application. Dale did not observe this action, perhaps because his method was too rigid and he also found phthaloyl-T and proto-catachoyl-T devoid of action; Marshall (12) states that the last paralyzes the vagus when injected intravenously.

Some of these simpler benzine tropeines thus have the characteristic atropine action though in a very low degree, none of them approaching the natural base nearer than in the ratio of 1:250 or 1:300; and from this ratio, which may be taken as the limit of comparison, others of the series descend until, as in the case of *p*-oxybenzoyl-T, there is difficulty in detecting any action whatever, except by the very sensitive reaction of the iris; and perhaps some of them are entirely inactive.

It may be doubted whether the distinction which has been drawn between the aliphatic and the aromatic tropeines is justified in its absolute form. For a very feeble action is claimed for some of the aliphatic series, and some of the aromatic tropeines have an equally weak effect. In some of the latter, the action is less equivocal, but the difference between the simpler aromatic tropeines and the aliphatic ones seems rather quantitative than qualitative.

In this relation, it may be remarked that the size of the acyl group does not seem to determine the activity. This is apparent from the difference in the reactions of the three oxybenzoyl-tropeines and also from the activity of the benzoyl-T, as compared with terebyl-T or phthaloyl-T.

The activity of all the tropeines I have examined is given in the following table, that of atropine being taken as 300.

<i>l</i> -hyosecyamine.....	600
Methyl-atropine.....	450
Atropine.....	300
<i>d</i> -hyosecyamine.....	15
<i>l</i> -homatropine.....	14
<i>dl</i> -homatropine.....	10
<i>d</i> -homatropine.....	7
Phenylacetyl-T.....	1
Benzoyl-T.....	1
<i>o</i> -oxybenzoyl-T.....	1
<i>m</i> -oxybenzoyl-T.....	<1
<i>p</i> -oxybenzoyl-T.....	<1
<i>d</i> -tartryl-T.....	0

In this table a sudden change is seen on passing from the tropeines of the simpler aromatic acids to the homatropine isomers, and this is the more remarkable when it is considered that among the former is phenylacetyl-tropeine ($C_6H_5CH_2COOT$), which differs from homatropine ($C_6H_5CHOHCOOT$) only in the latter containing an OH group in the side chain. The presence of this hydroxyl, however, renders one carbon asymmetric and the question arises whether the increase in action seen in passing from phenylacetyl-T to homatropine is due to the -OH directly or to the asymmetric carbon. Some light on this may be derived from the table given by Pyman, though no exact comparisons of the pharmacological activity are given in it; I have added such information as can be gathered from the text; only those items that bear on the question at issue are given and the tropeines with asymmetric carbon are indicated by italics:

Phenylacetyl-T, $PhCH_2COOT$less active than homatropine
Homatropine $PhCHOHCOOT$less active than homatropine
Phenylchloroacetyl-T, $PhCHClCOOT$less active than homatropine
Phenylaminoacetyl-T, $PhCHNH_2COOT$less active than homatropine

$$\begin{array}{c} CO-O \\ | \quad | \\ Phthalidicarboxyl-T \quad C_6H_4-CH-COOT \end{array}$$

.....less active than homatropine

Here the three last bases differ from homatropine only in having Cl, NH_2 , or O-CO- instead of the OH in the side chain, and

in each there is of course an asymmetric carbon. All three are stated to be less mydriatic than homatropine, and in fact are classed with phenylacetyl-T. If this estimate is correct, it would appear that while the presence of asymmetric carbon is of significance in intensifying the action of this group, as is shown by the comparison of *d* and *l* homatropine, its full effect is obtained only when it is accompanied by OH.

Among the tropeines related to atropine rather than to homatropine, the direction of optical rotation, that is the presence of an asymmetric carbon, again plays an important part in determining the pharmacological activity, and it is of interest to consider whether here also this has any relation to the presence of OH. The tropyl-tropeines contain both features, but differ from homatropine in not owing their asymmetry to the presence of hydroxyl. In the list of tropeines given by Pyman are some which might have decided the relative importance of asymmetry with OH and asymmetry without it, but unfortunately the estimations of the three most crucial instances by Lewin and Guillery (17) are vague and unsatisfactory, and the activity of hydratropyl-T ($\text{Ph-CH}(\text{CH}_3)\text{COOT}$) has not been determined. In the list I have inserted the values cited by Pyman which however must be taken as mere approximations. Class 1 indicates activity of the order of atropine, Class 2 of homatropine and Class 3 lower than homatropine.

Atropine $\text{Ph-CH}(\text{CH}_2\text{OH})\text{COOT}$	I
Acetyl-tropyl-T, $\text{Ph-CH}(\text{CH}_2\text{OCOCH}_3)-\text{COOT}$	I-II Lewin and Guillery
Chlorohydratropyl-T, $\text{Ph-CH}(\text{CH}_2\text{Cl})-\text{COOT}$	II Lewin and Guillery
Bromohydratropyl-T, $\text{Ph-CH}(\text{CH}_2\text{BN})-\text{COOT}$	II Lewin and Guillery
Atroglyceryl-T, $\text{Ph-COH}(\text{CH}_2\text{OH})-\text{COOT}$	I-II (Dale)
Atrolactyl-T, $\text{Ph-COH}(\text{CH}_3)-\text{COOT}$	II (Völkers) (18)
Atropyl-T, $\text{Ph-C}(\text{:CH}_2)-\text{COOT}$	III (Kobert) (19)
Cinnamoyl-T, $\text{Ph-CHCH}-\text{COOT}$	Inactive (Dale)
Phenylthioxypropionyl T, $\text{Ph-CH}_2\text{CHOH}-\text{COOT}$	I (Dale)

In this table all the substances contain an asymmetric carbon except two, atropyltropeine and cinnamoyl-tropeine, which are also devoid of hydroxyl in the side chain. Of these atropyl-tropeine (belladonnine) was said by Lewin and Guillery not to be mydriatic, but Kobert on more careful examination estimates

it as at least one hundred times as weak as hyoscine; so that it may be of the same order as benzoyl tropeine. It is of interest to find a tropeine, which is so closely related to atropine, reverting to this order of activity with the loss of the asymmetric carbon and hydroxyl. A similar descent in the scale of activity with exactly the same change in form is seen between cinnamoyl-T and phenyl-hydroxy-propinoyl-T.

The three tropeines which differ from atropine only in the substitution of Cl, Br and acetyl for OH have been assigned a distinctly lower activity by Lewin and Guillery whose method was crude. If their results are to be regarded as quantitatively valid, they would indicate that the OH here again enhances the activating action of the asymmetric carbon, even when it is not directly attached to it, as is the case of homatropine. Further and more exact measurements of the relative activity of this series are desirable and might well commence with hydratropyl-T, in which the asymmetric carbon is present without hydroxyl.

A number of other tropeines examined by Jowett and Pyman do not need discussion here, such as methyl-atropine, methyl-homatropine or methylmandelyl-T; they conform in type and in activity to the parent tropeines. Atropine-sulphuric acid and homatropine-sulphuric acid are devoid of action as they are not alkaloids but acids, and have a different distribution in the blood and tissues. Hippuryl-T ($\text{C}_6\text{H}_5\text{CONHCH}_2\text{COOT}$) is stated to be feebly mydriatic (Gottlieb) and phenylcarbamo-T was found to be of the same order of activity by Dale. These may perhaps be classified with the oxybenzoyl tropeines in degree of

activity. Iso-coumarincarboxyl-T $\left(\begin{array}{c} \text{C}_6\text{H}_4-\text{CH}-\text{C}-\text{COOT} \\ | \qquad \qquad | \\ \text{CO} \text{---} \text{O} \end{array} \right)$ was

found inactive by Symons (Pyman) and contains neither hydroxyl nor asymmetric carbon. On the other hand, the lactone

of *o*-carboxyphenyl-glyceryl-T $\left(\begin{array}{c} \text{CO} \text{---} \text{O} \\ | \qquad \qquad | \\ \text{C}_6\text{H}_4 \cdot \text{CHOH} \cdot \text{CH}-\text{COOT} \end{array} \right)$ was

also found inactive by him although it contains OH and two asymmetric carbons. This is the one example in all of the aro-

matic tropeines in which the presence of OH and asymmetric carbons is unable to give some degree of pharmacological activity. Among the aliphatic tropeines another example resembling the lactone of *o*-carboxyl-phenyl-glyceryl-T in possessing two asymmetric carbons is found in methyl-paraconyl-T, which is stated by Marshall and Dale to be inactive.

GENERAL CONCLUSIONS

Some general conclusions seem to be permissible from these observations. While tropine itself is devoid of the typical atropine action, many of its compounds, especially those containing the benzene nucleus, possess the characteristic action in some degree. This is greatly intensified by the presence of hydroxyl and of asymmetric carbon in the side chain, and the highest degree of activity is reached only when tropeine is combined with an acid of the benzene series, which contains a hydroxyl and an asymmetric carbon in the side chain, the whole molecule being laevorotary.

Even the dextrorotary isomer is considerably more active than the nearest homologues which do not possess the asymmetric carbon, and it might be supposed that the presence of the latter in itself has a certain intensifying action whatever the sign of the rotation. This cannot be definitely asserted however, because in the instances examined by me, the asymmetric carbon was always accompanied by OH, and this appears to intensify the action in some degree. The greater activity of the *d*-hyoscyamine and of *d*-homatropine as compared with phenylacetyl-T may arise from their containing the OH group. It would be of interest to determine accurately the power of *d*-phenylechloracetyl-T and other similar substances, which contain the asymmetric carbon without OH, and in particular to compare them with phenylacetyl-T. This I have not been able to do, and the only statement to be found on the subject is that of Pyman, who says that the (racemic) forms are less powerful than *dl*-homatropine as mydriatics.

In considering the relative activity of nearly connected substances, the importance of the presence of asymmetric carbon has not hitherto been recognized.

Another series in which it appears to play a part, and in which the strength of the members has been carefully measured, is formed by the adrenaline (sympathomimetic) bodies which were compared by Barger and Dale (21). In this series there is again, as in the tropeines, some inherent action in a large series of amines, including aliphatic amines, though these are weaker than the aromatic ones. Among the aromatic amines without phenolic hydroxyl the presence of an asymmetric carbon does not seem to increase the activity noticeably, even when the asymmetry is due to the presence of a hydroxyl group in the side chain, but when phenolic hydroxyls are present, the occurrence of an asymmetric carbon with hydroxyl attached raises the activity in one instance 50 times, in another 7 times; the relative activity of amino-ethyl-catechol, $(\text{OH})_2\text{C}_6\text{H}_3\text{CH}_2\text{CH}_2\text{NH}_2$, and aminoethanolecatechol, *dl* $(\text{OH})_2\text{C}_6\text{H}_3\text{CHOH}\cdot\text{CH}_2\text{NH}_2$ is 1:50; that of methylamino-ethyl-catechol, $(\text{OH})_2\text{C}_6\text{H}_3\text{CH}_2\text{CH}_2\text{CHNH}_2$; and methylamino-ethanol-catechol, *dl* $(\text{OH})_2\text{C}_6\text{H}_3\text{CHOH}\cdot\text{CH}_2\text{NHCH}_3$, is 1:7. I have shown that in the latter example (adrenaline) the *l* form is 12 to 15 times as powerful as the *d*, so that the toxicity of the *dl* form must be 6 to 7 times that of the *d*. The increase in the activity found by Barger and Dale in passing from the methylamino-ethyl-catechol to the *dl* adrenaline is wholly due to the presence of the laevo-rotary component, and thus to the asymmetric carbon.

In the tropeine series, the highest activity is attained only when asymmetric carbon is associated with hydroxyl, and it is tempting to consider the former as an anchoring or haptophoric group and the hydroxyl as bearing some such relation to the molecule as Ehrlich designated by the toxophore. The fact that tropeines which contain neither of these factors still have some action on the receptors is opposed to this however, and the activating power of the hydroxyl may probably arise from some physical property with which it endows the whole molecule; compare the difference in physical properties in such molecules as ethane and alcohol.

The influence of the asymmetric carbon in determining the activity in accordance with the direction of optical rotation is a special case of Fischer's lock and key reactions, and is best explained on the analogy of the reaction of these isomers with other optically active substances. In each case the more marked effect of one of the isomers probably arises from its compound formed with the receptors of the tissues possessing physical properties, such as solubility, different from those of its isomer of the opposite sign.

ADDENDUM

Methylatropine

The quaternary base, methylatropine, was first examined by Brown and Fraser (6), who found the curare action greater than that of atropine, while the mydriatic action was of the same

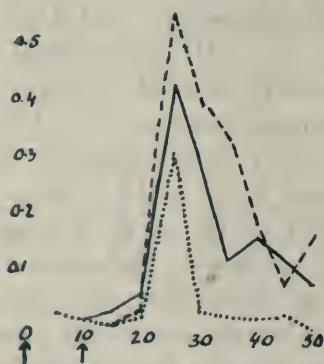


FIG. 10. Graphs of pilocarpine secretion after the injection of 0.1 mgm. atropine (—), 0.1 mgm. methyl-atropine (· · ·), and 0.05 mgm. methylatropine (- - -) respectively.

order. Erbe (7) found that the minimal amount required to dilate the pupil is practically the same as that of atropine, and clinically methylatropine (or eumydrine) has been found rather weaker and shorter in its mydriatic action than atropine (Erbe (7), Lindenmeyer (8), Goldberg (9). More recently Issekutz (10) has compared the action of the quaternary bases of atropine and homatropine with the parent alkaloids and finds that methyl-

atropine acts about 8 times as strongly as atropine on the frog's vagus, twice as strongly in antagonizing pilocarpine in the excised bowel and 3 to 4 times as strongly in the salivary gland; they seem to be of equal power as mydriatics. His methods are not very delicate, and I have therefore controlled his results on the salivary secretion by my usual method. The sample of methylatropine bromide used had a melting point of 222°C. and was therefore chemically pure. I found atropine 0.1 mgm. weaker than methylatropine 0.1 mgm., but stronger than 0.05 mgm. (fig. 10). Methylatropine may thus be taken as about $1\frac{1}{2}$ times as powerful as atropine, each calculated as base.

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THE ACTION OF DRUGS UPON THE OUTPUT OF EPINEPHRIN FROM THE ADRENALS I. STRYCHNINE

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We have already more than once emphasized the fact that the spontaneous liberation of epinephrin is not easily influenced by experimental conditions. The relatively great stability of the rate of output made it likely that if drugs are capable of affecting the output in any marked degree there would be no special difficulty in detecting and estimating the amount of the change. We began with strychnine, because it seemed probable that a drug which caused so marked an effect on the motor mechanisms of the cord would also exert an action upon the mechanism which is associated with the liberation of epinephrin from the adrenals. We had previously found that the injection into the blood stream of small quantities of concentrated solutions of salts (sodium carbonate), which, as is known, causes a general excitaton of the cord, with convulsions, was followed by a transient but marked increase in the epinephrin output.

DISCUSSION OF ESSENTIALS IN THE TECHNIQUE OF MEASURING THE EPINEPHRIN OUTPUT

Many of the statements in the literature upon the effect of drugs and other substances on the epinephrin output are based upon experiments by inadequate methods. It is not merely that some writers habitually interpret changes in the epinephrin store of the adrenals in terms of changes in the rate of liberation of epinephrin, but even when it has been recognized that it is the changes in the blood coming from the gland

which it is essential to investigate it has often been strangely overlooked that when the concentration of epinephrin in the drawn blood has been estimated it is still necessary to know the rate of the blood flow through the adrenals in order to arrive at the rate of output. How little this matter, elementary as it may seem, has been understood, appears from the fact that one of the methods most frequently employed to study changes in the epinephrin output (collection of blood by a catheter from the inferior cava above the level of the adrenal veins (1), (2), and subsequent application of the blood to intestine strips or segments) does not permit the estimation of the flow through the adrenals or even in the cava. We say nothing of the fact that the adrenal blood is diluted greatly and probably in a different degree in successive observations, with the ordinary venous blood. Even those investigators who have recognized that the concentration of epinephrin in adrenal vein blood being at best small it is desirable to work with pure adrenal blood, have not always realized that the rate of flow is an indispensable factor for the determination by such methods of the rate of output. Gley and Quinquaud, for example, in a recent paper (3) remark that in asphyxia the output of epinephrin is increased, since they have found that two or three times as much adrenal blood collected from a dog, without asphyxia, must be injected into another dog to cause a given rise of blood pressure as of adrenal blood collected during asphyxia lasting two to four minutes. That they have taken no account of the rate of the blood flow is obvious since they bring forward as confirmatory evidence of an increased output the fact that a given amount of blood collected from the inferior cava above the adrenals without asphyxia causes a smaller rise of pressure in another dog than an equal amount collected during asphyxia. Here they could not possibly have measured the rate of flow.

They conclude, nevertheless, that the quantities of epinephrin liberated are too small to cause any demonstrable reaction in the organism, since they cannot detect epinephrin in the blood of the right heart even when the output is increased by stimulation of the peripheral end of the splanchnic nerve. We believe that these writers are mistaken in both conclusions: asphyxia, at any rate for such periods and with such methods as we have employed, does not produce a demonstrable increase in the output of epinephrin, but if it did definite reactions could easily be caused by it. It has been shown, as a matter of fact, by Joseph and Meltzer (4) that stimulation of the splanchnic causes dilatation of the pupil on the side on which the superior cervical ganglion has been previously

excised. Their interpretation of this dilatation as due to increased epinephrin liberation from the adrenals has been abundantly confirmed by Elliott (5), Stewart, Rogoff and Gibson (6), and others. We have even obtained evidence that the amount of epinephrin spontaneously liberated is not without effect upon the denervated iris and other structures (7). The only reason for the failure of Gley and Quinquaud to demonstrate epinephrin in the blood of the right heart is that the reaction used by them (the rise of blood pressure produced in one dog by injection of blood from another) although in other respects a good reaction, is not delicate enough.

Their supposed proof of an increased output of epinephrin in asphyxia is nothing more than a proof of an increased concentration of epinephrin in the adrenal vein (or cava) blood. Such an increased concentration would necessarily occur, if the rate of output per minute remained unaltered, provided that the average blood flow through the adrenals (or in the cava) was diminished by prolonged asphyxia. This is precisely what we have found to occur, as shown in the following experiment.

Condensed protocol; dog 307; female; weight, 4.6 kgm.

Anesthetized with morphine and ether. Obtained a specimen of indifferent blood from external jugular vein. Made cava pocket. Then collected adrenal blood.

- 11.44 a.m. First specimen, 4.25 grams in 30 seconds (8.5 grams per minute).
- 11.44½ a.m. Second specimen, 11.05 grams in 90 seconds (7.4 grams per minute). Blood pressure during collection of adrenal specimen was 90 mm. Hg.
- 11.50 a.m. Total asphyxia started. Then collected adrenal blood.
- 11.50½ a.m. Third specimen, 2.9 grams in 60 seconds (2.9 grams per minute).
- 11.51½ a.m. Fourth specimen, 5.75 grams in 90 seconds (3.8 grams per minute). Blood pressure towards end of collection of fourth specimen 72 mm. Hg.
- 11.53 a.m. Stopped asphyxia for a short period (20 to 30 seconds).
- 11.53 a.m. Fifth specimen, 9.2 grams in 120 seconds (4.6 grams per minute).
- 11.53½ a.m. Started total asphyxia.

- 11.55 a.m. Sixth specimen, 7.6 grams in 120 seconds (3.8 grams per minute). Blood pressure during collection of sixth specimen 62 mm. Hg.
- 11.57 a.m. Started artificial respiration.
- 12.05 p.m. Seventh specimen, 9.45 grams in 120 seconds (4.7 grams per minute).
- 12.07 p.m. Eighth specimen, 9.75 grams in 120 seconds (4.9 grams per minute). Blood pressure during collection of eighth specimen 60 mm. Hg.
- 12.08 p.m. Started total asphyxia.
- 12.10 p.m. Ninth specimen, 3.5 grams in 120 seconds (1.8 grams per minute). Blood pressure during collection of ninth specimen 56 mm. Hg.
- 12.12 p.m. Started artificial respiration and obtained abdominal aorta (indifferent) blood.
- Combined weight of adrenals 0.831 gram.



FIG. 1. INTESTINE TRACINGS. BLOOD FROM DOG 307

At 1, 3, 5, and 7 Ringer was replaced by indifferent (jugular) blood, and this at 2, by the second adrenal specimen (collected before asphyxia); at 4 by the sixth specimen (collected during and after prolonged asphyxia); at 6 by the eighth specimen (collected eight minutes after end of asphyxial period); at 8 by the second specimen. All the bloods were diluted with three volumes Ringer. As in all figures showing intestine or uterus tracings, time is marked in half minutes. (Reduced to one-half.)

The second adrenal specimen (taken before asphyxia) had a smaller concentration of epinephrin than the sixth (taken during and after prolonged asphyxia). The eighth specimen (collected after artificial respiration had been going on for some time) was intermediate in strength. This is well illustrated in figure 1. At this stage the intestine segment

was not very sensitive to epinephrin, so that little more than a just detectable reaction was given by the second specimen (observation 2) the first time it was applied to the segment. A little later the second specimen gave a somewhat greater reaction (observation 8). A reader unfamiliar with these methods of assay might have supposed from a simple comparison of observations 2 and 4 that the output of epinephrin must have been increased when the asphyxia specimen was being collected. Indeed, some of the statements in the literature to this effect are based on no better evidence. The truth is, of course, that here the sensitiveness of the segment was such as just to permit the detection of the epinephrin concentration in the second specimen. Had the segment been a little less sensitive, the second specimen would have given no

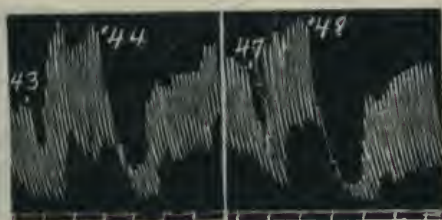


FIG. 2. INTESTINE TRACINGS. BLOODS FROM DOG 307

At 43 and 47 Ringer was replaced by indifferent (jugular) blood, and this at 44 and 48 by the second and sixth adrenal specimens (collected before and during prolonged asphyxia) respectively. The bloods were diluted with three volumes Ringer. (Reduced to one-half.)

reaction, while the sixth would have given a good reaction and the conclusion might have been drawn by a novice that an outburst of epinephrin had occurred during the period of asphyxia. The assay showed, as a matter of fact, that the output was not at all augmented. Later on, the sensitiveness of the segment being increased, the reaction caused by the second specimen was much greater, but the preponderance of the sixth was still maintained (fig. 2, observations 44 and 48). The eighth specimen also occupied the same relative position, being weaker than the sixth (fig. 3, observations 54 and 60). The ninth specimen, collected during a further period of asphyxia and with a much smaller blood flow than any of the others, had, of course, the greatest concentration of all (fig. 3, observation 56).

Observations on a uterus segment demonstrated that the various adrenal specimens caused increases in the tone of the preparation cor-

responding to the inhibitory effects produced on the intestine segment, indicating that the inhibition was due to epinephrin and not to other substances said sometimes to be present in the general blood stream in asphyxia which cause a diminution of tone in both rabbit intestine and uterus.

The epinephrin assay showed that the second adrenal specimen (collected before asphyxia) was much weaker than 1:1,500,000 adrenalin, weaker than 1:3,000,000, a little weaker than 1:4,500,000, much stronger than 1:7,500,000, decidedly stronger than 1:6,000,000, probably somewhat stronger than 1:5,200,000. It was finally taken at 1:4,800,000, corresponding to an output of 0.0015 mgm. per minute for the dog, or 0.00033 mgm. per kilogram per minute.

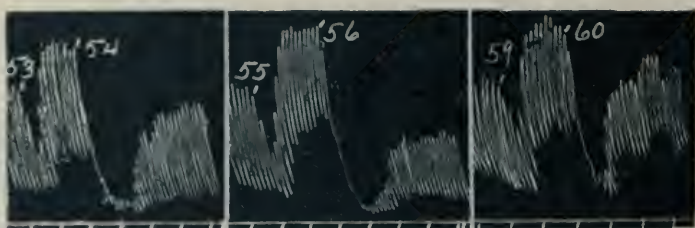


FIG. 3. INTESTINE TRACINGS. BLOOD FROM DOG 307

At 53, 55, and 59 Ringer was replaced by indifferent (jugular) blood, and this at 54 by the sixth adrenal specimen (asphyxia); at 56 by the ninth (asphyxia); at 60 by the eighth specimen (collected eight minutes after end of an asphyxial period). The bloods were all diluted with three volumes Ringer. (Reduced to one-half.)

The sixth specimen (collected during and after prolonged asphyxia) was decidedly weaker than 1:1,500,000, and very nearly equal to 1:2,200,000, probably slightly weaker. Taking it at 1:2,300,000 we get 0.0016 mgm. per minute for the dog or 0.00035 mgm. per kilogram per minute, the same as for the second specimen.

The eighth specimen (collected after a period of artificial respiration) was assayed at 1:3,000,000, corresponding to an output of 0.0016 mgm. per minute for the dog, or 0.00035 mgm. per kilogram per minute.

The ninth specimen (collected during asphyxia) was found to be weaker than 1:1,100,000, a little stronger than 1:1,500,000. It was taken as 1:1,300,000, corresponding to an output of 0.0014 mgm. per minute for the dog or 0.0003 mgm. per kilogram per minute. Obviously, notwithstanding the great increase in the concentration, the out-

put remained unchanged during asphyxia. The proportion of serum was 62 per cent, determined by the electrical method (8). The concentration of epinephrin in the serum of this specimen was accordingly 1:800,000, considerably less than the "possible normal maximum."¹

We have previously shown (9) that asphyxia does not increase the output, although when pushed to the point at which it diminishes the adrenal flow, it increases the concentration of epinephrin in the adrenal blood. A slowing of the blood flow produced in any other way, as by hemorrhage, inhibition of the heart by vagus stimulation, impairment of the heart or of the vasoconstrictor mechanism is associated with a similar increase in the concentration of epinephrin in the adrenal vein blood. From an increased concentration nothing whatever can be deduced as to an increase in the rate of output of epinephrin, unless it is known that the blood flow has not been correspondingly decreased. The matter is precisely on the same footing as the measurement of the rate of production of carbon dioxide by a tissue or organ, by determining the carbon dioxide content of the incoming and outgoing blood. Who would conclude from the fact that the carbon dioxide content of venous blood coming from the hind legs of an animal was increased relatively to that in the arterial blood, when the arterial pressure was lowered by stimulation of the vagus or by such a poison as nicotine, that the metabolism of the hind legs had been increased by the stimulation of certain fibres in the vagus or by the poison? Would it not be quite clear that the carbon dioxide production per minute had not been determined by estimating merely the carbon dioxide content of the blood and that in addition a measurement of the rate of blood flow was necessary? In this case it would be found that the increase in the excess of carbon dioxide content of venous as compared with arterial blood was just balanced by the decreased blood flow and that there was no increase in the output. To conclude from an observed increase in the epinephrin concentration in the blood coming from the adrenals that the output per minute has been increased, without information as to changes in the rate of blood flow is quite as unjustifiable as to come to a similar conclusion in regard to carbon dioxide. Whether epinephrin is a

¹ By the "possible normal maximum concentration" is meant the maximum concentration of epinephrin (assayed by rabbit's intestine and uterus segments), actually observed in the blood or serum in adrenal blood specimens collected with the slowest blood flows in animals anaesthetised with morphine, ether, or urethane. When this maximum concentration has been reached it is obvious that the output of epinephrin cannot be increased unless the blood flow is increased.

product of the metabolism of the adrenal medulla which is simply being got rid of, or a secretion with a function in the organism has, of course, nothing to do with the question.

It is the same with the experiments from which Gley and Quinquaud (*Arch. internat. de Physiol.*, 1914, xiv, 152) deduce the conclusion that relatively large doses of extracts of thyroid and other glands (pancreas, liver, etc.) increase the output of epinephrin when injected intravenously. These extracts are known to lower the blood pressure, and a figure is even given by the French authors showing this in the case of an injection of liver extract. The adrenal blood flow may, therefore, be assumed to have been diminished during collection of the sample of blood which is supposed to demonstrate the increased epinephrin output, and the concentration of epinephrin in it must have been increased. Yet because a given quantity of this blood produces a greater rise of pressure in another dog than an equal quantity of the "normal" adrenal blood it is concluded that the rate of output of epinephrin has been increased by the organ extract. Quinquaud (*Trav. du lab. de biol. gén. de Collège de France*, 1915)—has been misled by want of attention to the same point in his conclusion that piqure increases the epinephrin output.

It ought to be emphasized that the method used by these observers is adequate for determining the concentration of epinephrin in the adrenal vein blood. The results are vitiated merely by the failure to take account of the rate of adrenal blood flow. Quantitative conclusions have, however, been drawn not infrequently from methods which are unsuited to yield quantitative information.

Quite recently Kellaway (*Journ. of Physiol.*, 1919, lii, 63) has stated that "the paradoxical reaction of the pupil to asphyxia is wholly due to anoxaemia and mainly to . . . accelerated secretion from the adrenal glands." Even granting that anoxaemia produces constantly a much smaller dilatation of the pupil (sensitized by previous removal of the superior cervical ganglion) when the adrenals have been excised or the splanchnic nerves cut than with intact adrenals, all that ought to be deduced from this is that the epinephrin coming from the adrenals is concerned in the dilatation of the "sensitized" pupil. The epinephrin discharged at the ordinary rate is known to exert an action on the sensitized pupil and there is every reason to expect that asphyxia, which even according to Kellaway causes some dilatation in the absence of the adrenals, will increase the reactivity of the pupil to this ordinary output. Changes in the circulation

associated with asphyxia may also increase the blood flow through the sensitized iris and therefore the amount of epinephrin passing through it per unit of time. Such circulatory changes may also alter the concentration of epinephrin in the inferior cava without any alteration whatever having occurred in the rate of output. All these sources of error are eliminated by methods which permit the collection of adrenal vein blood and its assay on test objects which have not themselves been subjected to such conditions as asphyxia.

As a matter of fact, the response of the sensitized pupil to asphyxia varies quantitatively to a considerable extent in different cats after interference with the epinephrin discharge and also varies according to the degree of the asphyxia. But this is also true of normal cats, and we have never been able to convince ourselves that there is any striking difference. It may be that a very large series of observations would bring out some difference and this we should rather expect if the normal epinephrin output is already exerting an action. Possibly also the more gradual diminution of the oxygen in the blood when an animal is caused to breathe a gaseous mixture with a constant although diminished oxygen content, may cause a somewhat different effect from that of total asphyxia suddenly produced. We are certain, however, that one or two observations possess no value in a question of this kind. As already stated, such observations are in any case quite unsuitable for determining whether changes in the rate of epinephrin output are occurring or not. When we began our work on asphyxia it was with the expectation that so general an excitant of nervous centers would be found to excite the epinephrin secretory mechanism. But hitherto we have never been able to demonstrate such an action. Whether in an animal dying in asphyxial convulsions the central mechanism governing the liberation of epinephrin might be stimulated along with other centers with a resultant increase in the rate of epinephrin output, if the adrenal medulla was still capable of responding to the stimulation, is unknown.

An obvious fallacy underlies the conclusion of Roger (J. de Physiol. et Path. gén., 1917, xvii, 187) that because the cardiac inhibition, produced by stimulating the peripheral end of the cut vagus is less durable in normal than in adrenalectomized rabbits, in the former the inhibition must be cut short by the action of an *augmented* epinephrin discharge upon the heart. The observations, assuming their accuracy, can only show at most that the epinephrin coming from the adrenals exerts an action upon the heart which terminates the in-

hibition sooner than when epinephrin is absent. They may constitute, indeed, a proof that the normal output of epinephrin exerts a demonstrable effect upon the heart, of which we possess other proofs. But they cannot possibly demonstrate an *increased* output.

Technique. Blood was collected in the usual way from a cannula inserted into a pocket of the inferior cava. In making the pocket all veins entering the cava below the level of the liver, except the adrenal veins were tied. We always perform the operation in such a way that no leakage of any blood takes place into the pocket, except from the adrenals. In our experiments we are certain that only pure adrenal blood was collected, both in dogs and cats. The abdominal aorta was ligated near the bifurcation. The renal with the spermatic or ovarian arteries were also tied. In collecting the adrenal blood samples, a preliminary specimen was taken first, in order to wash out any epinephrin possibly liberated in the manipulation. The total dead space in cannula and vein in a cat is about 0.5 to 1 cc. In the rather small dogs employed in these experiments the dead space is about 1 to 2 cc. A fresh cannula (boiled and oiled) is always inserted after the collection of each pair of specimens. The cannula and vein are empty at the moment when the pocket is clamped off above the level of the right adrenal vein, and the time of collection of the first specimen is reckoned from the moment when blood begins to drop into the dish. The true time at which the blood began to leave the adrenals is obtained approximately by correcting for the blood required to fill the dead space at the given rate of flow. This is only necessary when it is required to fix more exactly the time interval between the introduction of a new factor in the experiment, for example, the injection of a drug, and the beginning of the resultant change in the epinephrin secretion.

The blood specimens were defibrinated and kept on ice till the assay on rabbit intestine (and uterus) segments was made. The assay was begun in general within an hour of obtaining the last specimen. In a very few instances it was necessary to leave the completion of the assay of one or two of the samples till the following morning. When this was inevitable a provisional assay was made on the day on which the blood was drawn. Dale and Laidlaw (10) have asserted that defibrinated blood is not suitable for assaying epinephrin on intestine strips or segments, because of the presence of a substance developed in clotting which exerts a tone-increasing effect on the unstriped muscle. They recommend the injection of hirudin into the blood to prevent clotting. We have no objection to the use of hirudin to prevent clots in the can-

nula, although we believe it is better to insure against this by inserting fresh cannulae and avoid injection of hirudin at least until it is proved that it has no effect on the epinephrin output. Also hirudin itself is capable of causing some effect on the test segments. But we are certain that it is an error to suppose that it is a disadvantage that the blood should cause an increase of tone in the segment. We believe, on the contrary, that it is a distinct advantage, as the tone-decreasing power of the epinephrin-containing blood when it displaces the indifferent blood has a better opportunity of manifesting itself when the tone has been previously increased. In our work we prefer that the indifferent blood should cause a good increase of tone in the segment, and we have often observed that a segment which at first responded to the blood by only a small increase of tone or none at all, and was not very sensitive to epinephrin, became decidedly more sensitive later on when its tone was increased to a greater extent by the indifferent blood.

In any case, as was shown by Stewart (11) and by Stewart and Zucker (12) the difference in the action of plasma and serum is much more conspicuous, more constant and more easily elicited in the case of vascular objects, like artery rings or perfused frogs' legs than in the case of rabbit intestine or uterus segments. With undiluted or only moderately diluted heterologous blood, little if any difference was found in the tone-increasing effect of the unclotted and defibrinated blood, or of the plasma and serum. The main object of that work was to determine a question of considerable importance in the technique of testing for epinephrin in blood, namely whether any advantage was to be gained by using unclotted blood or plasma for application to rabbit intestine and uterus segments, rather than the much more easily obtained defibrinated blood or serum. For this reason, only such kinds of blood (almost always heterologous) and such dilutions (never very great, especially in the case of the intestine) were employed as were most commonly used in the epinephrin determinations. It was clearly pointed out that our results "need not imply that the pressor substance, if it is a single definite substance developed in the shed blood, exerts no action on the smooth muscle of the intestine and uterus preparations, but merely that its action on these objects is masked by the general action of the serum and plasma, so that, in the presence of the other constituents common to serum and plasma, its effect is inconspicuous or not to be detected at all, while on the blood vessel preparations, especially the artery rings, the effect of the pressor substance is the dominant one, and the general action of the serum and plasma is feeble or undetectable."

It must be remembered that an intestine or uterus segment is a very complex structure compared with an artery ring. It would be nothing short of miraculous if a segment which shows itself so sensitive to slight changes in the composition of an artificial fluid in which it is beating, and to changes in the oxygen supply should be quite unaffected when Ringer's solution is replaced by such a liquid as hirudin plasma or unclotted hirudin blood. As a matter of fact, we found that such liquids as hydrocele fluid and ascitic fluid, which had never clotted and were incapable of spontaneous coagulation, invariably caused the same qualitative effects as serum on rabbit intestine or uterus segments, although, quite inert as regards artery rings. Serum which had lost its power of constricting artery rings (13) after digestion with blood vessels (Tatum (14)) and after being subjected to other processes still caused a marked increase in the tone of intestine and uterus segments. There is some reason to think that the specific action of the serum on smooth muscle may be more evident as compared with its general action on the segments when the serum is considerably diluted. We, ourselves, saw and have figured instances of this, as already stated. In most of our experiments the serum and plasma, or the defibrinated and unclotted blood were either undiluted or only moderately diluted and here the general action common to both preponderated. Where a small amount of blood is run into a relatively large vessel of Ringer's or Tyrode's solution, as was done by Dittler (15), the difference in the action of serum and plasma may, therefore, become more conspicuous than in our observations.

Another fact must be carefully kept in mind. We have several times pointed out that in general the first applications of blood or serum to a segment, especially to an intestine segment, will produce an increase of tone which is less, often much less, than it will be in subsequent applications. For this reason, in our epinephrin assays we never begin work with the adrenal bloods until the segment has been once or twice subjected to the action of the indifferent blood. If now, on comparing the action of serum and plasma, the plasma be added to a fresh segment, the effect will usually be decidedly less than that of serum when this is added later, or there may appear to be little, if any, effect. It is always necessary in such comparisons to make numerous observations and to vary the order in which the liquids are applied to the segment. This circumstance must also be taken into account in such experiments as those of Dittler, in which arterial blood is run directly into the vessel where a fresh segment is beating in the artificial saline medium. It may

be expected that the period which elapses before the segment reacts will be longer for this reason if for no other, than when in a subsequent observation, defibrinated blood is similarly applied to the segment. It is not suggested that the whole of the difference between unclotted and clotted blood observed by Dittler can be explained in this way, but this factor must be taken into account.

It must further be considered that in Dittler's experiments the unclotted blood was always applied to intestine segments from the same animal, while this was not always the case with the serum or defibrinated blood. In our experiments indications were observed that differences between plasma and serum (or unclotted and defibrinated blood) were more likely to exist and to be of greater magnitude in the case of homologous than in the case of heterologous blood. As the latter has been invariably employed in our epinephrin assays, this is an additional reason for not attempting at the cost of complicating the experiments considerably to gain a theoretical advantage by using unclotted instead of defibrinated blood. A more important contraindication is that even if it were admitted that when great precautions are taken unclotted blood can be obtained from other animals as well as from rabbits, which in considerable dilution will be without action on rabbit segments, there is no guarantee that this blood will not clot and develop its pressor effect in a manner quite beyond control in contact with the segment.

O'Connor (16) indeed, who maintains that the tone-increasing action of blood on the intestine is entirely developed in clotting says that this is very difficult to demonstrate for the precise reason that in the presence of the thromboplastic substances in the intestine segment clotting may occur. If this were true, it is obvious that with the use of plasma in such long series of observations as are practised by us in assaying epinephrin in adrenal blood, the reaction of the segment to the indifferent blood and, therefore, the extent of the inhibition produced by the epinephrin-containing blood might be expected to vary in a manner which could not be controlled in successive observations. On the other hand, the increase of tone produced by a specimen of defibrinated blood is sufficiently constant to allow numerous comparative observations to be made on the same segment.

As in our experience, rabbit intestine (and uterus) segments constitute the best objects at present known for the assay of epinephrin in blood, we have entered at some length into the question, probably the most important in the whole technique, whether it is necessary to use unclotted blood or plasma. We have no hesitation in concluding that far

from being necessary, it is not advantageous to attempt to do so. Defibrinated blood (or serum) is better. Writers who have suggested that the results obtained on intestine segments or strips were vitiated by the fact that defibrinated blood or serum was employed have not themselves had any experience of the method, and have been misled by the fact that for the vascular test objects the conditions are quite different. Thus, for tests with artery rings unclotted blood (or plasma) is indispensable, while for the frog perfusion preparation it is probably considerably superior to defibrinated blood (or serum).

We regret the necessity of discussing at such length these questions of technique. But most of the confusion and uncertainty in the literature of the subject is due to the misinterpretation of results obtained by methods which have been applied without adequate examination and criticism. A discussion of methods seems therefore an almost indispensable preamble to a series of papers purporting to examine the action of drugs upon the output of epinephrin. We return now to our experiments.

While in the case of strychnine and the other drugs examined we relied mainly on epinephrin assays with rabbit segments, corroborative evidence was sought: (a) By comparing the rise of blood pressure produced by adrenal blood collected for a definite time in a cava pocket and then released, before and after administration of the drug. (b) By studying the effects of the drug on the eye after excision of the superior cervical ganglion, in normal cats and in cats whose epinephrin output had been interfered with by removal of one adrenal and section of the nerves of the other.

The preference must always be given, we believe, to methods in which blood is collected directly from the adrenals and the epinephrin content of the drawn blood then assayed on suitable test objects. Next comes the method in which the adrenal blood is collected for a given time in a cava pocket and then released into the circulation of the animal, where it produces some definite effect as on the blood pressure or the structures of the denervated eye, the amount of which effect can be estimated. An obvious limitation of this method of auto-assay is that procedures whose effect on the epinephrin output is being studied may themselves alter the sensitiveness of the test objects.

The mere comparison in the intact animal of the effect of drugs on such objects as the denervated eye with the adrenals discharging epinephrin and when the discharge has been suppressed, can only be used in general to corroborate results obtained by more direct methods.

Observations of this kind must be interpreted with great care if deductions are to be drawn as to any effect produced on the epinephrin output. Many errors have arisen from the uncritical use of such observations, which at best can seldom yield reliable quantitative results.

A few experiments were made on the influence of some of the drugs on the epinephrin store of the adrenals.

EXPERIMENTS ON DOGS

We began purposely with large doses in order to produce a marked effect. Later on the effect of smaller doses was studied. No attempt is made in this paper to fix the minimum effective dose, which must in any case vary with the anaesthetic and other circumstances, but doses lying well within the therapeutic range were found to produce decided increases in the epinephrin output.

Some typical experiments on dogs, with condensed protocols and specimens of the tracings used in the epinephrin assay, will now be given. As for each animal, on the average, the epinephrin assay involved at least 30 to 40 separate applications to the segments, of adrenal blood or indifferent blood made up with adrenalin, it is obviously impossible to reproduce a sufficient number of tracings to give a complete picture of the assay in even one animal. We have, therefore, judged it best to illustrate pretty fully the assay in one experiment and for the others to give only enough tracings to bring out special points. In the experiment chosen the strychnine effect was neither the largest nor the smallest observed.

Condensed protocol; dog 257; female; weight, 6.0 kgm.

Anesthetized with morphine and ether. Obtained a specimen of indifferent blood from external jugular vein. Made cava pocket. Blood pressure 118 mm. Hg. Collected adrenal blood.

- 11.20 a.m. First specimen, 4.65 grams in 30 seconds (9.3 grams per minute). Second specimen, 8.65 grams in 60 seconds (8.65 grams per minute).
- 11.30 a.m. Injected 0.4 mgm, strychnine sulphate into jugular vein.
- 11.31 a.m. Reflexes exaggerated; no convulsions. Third specimen, 4.3 grams in 30 seconds (8.6 grams per minute). Fourth specimen, 8.8 grams in 60 seconds (8.8 grams per minute).

- 11.35 a.m. Blood pressure 114 mm. Hg.
 12.05 p.m. Blood pressure 96 mm. Hg. Fifth specimen, 4.35 grams in 30 seconds (8.7 grams per minute). Sixth specimen 7.95 grams in 60 seconds (7.95 grams per minute).
 12.15 p.m. Injected 0.5 mgm. strychnine into jugular vein. Marked clonic spasms.
 12.16 p.m. Seventh specimen, 2.8 grams in 30 seconds (5.6 grams per minute). Eighth specimen, 7.45 grams in 120 seconds (3.7 grams per minute).

During collection of the eighth specimen, tonic spasm occurred. Combined weight of adrenals 0.908 gram.

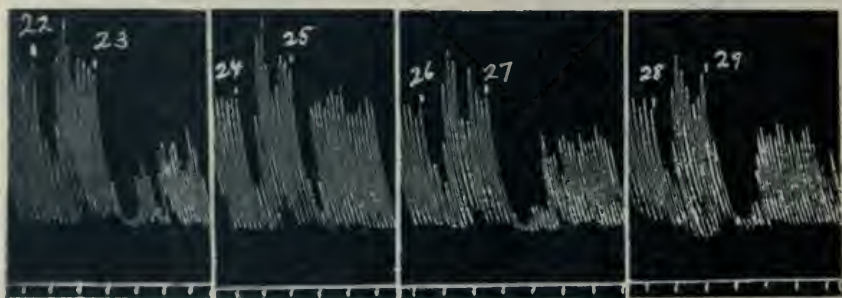


FIG. 4. INTESTINE TRACINGS. BLOODS FROM DOG 257

At 22, 24, 26, and 28 Ringer was replaced by indifferent (jugular) blood, and this at 23 by jugular blood made up with adrenalin to a concentration of 1:5,000,000; at 25 by jugular blood made up with adrenalin to a concentration of 1:8,300,000; at 27 by jugular blood made up with adrenalin to a concentration of 1:6,666,000; at 29 by the second adrenal specimen (collected before strychnine). All the bloods were diluted with three volumes Ringer, the adrenalin bloods after addition of the adrenalin. (Reduced to one-half.)

Tracings reproduced in figures 4 to 6 illustrate the assay of two of the adrenal specimens, the second, taken before the first injection of strychnine and the eighth, taken after a second strychnine injection. In figure 4 it is shown that the second specimen (observation 29) is much stronger than 1:8,000,000 (observation 25), decidedly weaker than 1:5,000,000 (observation 23), and not much different from 1:6,660,000 (observation 27).

Figure 5 indicates that the eighth specimen (observation 9) is much stronger than 1:3,300,000 (observaton 11), and figure 6 that it is stronger than 1:2,500,000 (observations 13 and 15), stronger than 1:1,660,000 (observations 15 and 17), and not very different from 1:830,000. It was confirmed by other observations (not reproduced) that the eighth specimen was decidedly stronger than 1:1,660,000, stronger than 1:1,250,000 and a little stronger than 1:830,000. Taking the second specimen at 1:6,700,000, we get 0.0013 mgm. per minute for the dog, or

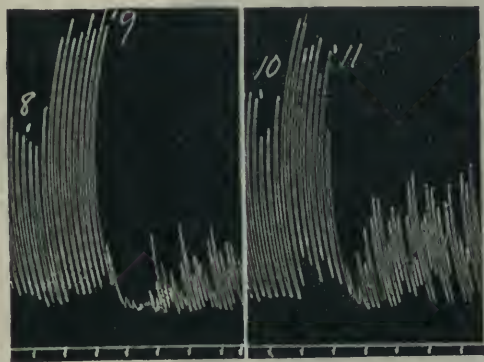


FIG. 5. INTESTINE TRACINGS. BLOODS FROM DOG 257

At 8 and 10 Ringer was replaced by indifferent (jugular) blood, and this at 9 by the eighth adrenal specimen (collected forty-five minutes after strychnine); and at 11 by jugular blood made up with adrenalin to a concentration of 1:3,300,000. The bloods were diluted with three volumes Ringer, the adrenalin blood after adding the adrenalin. (Reduced to one-half.)

0.0002 mgm. per kilogram per minute, the average output in dogs.

Taking the eighth specimen at 1:800,000, we get 0.0045 mgm. per minute for the dog, or 0.00075 mgm. per kilogram per minute. The output when the eighth specimen was collected was, therefore, nearly four times as great as before the first strychnine injection. The first dose of strychnine in this experiment only caused some exaggeration of the reflex excitability, but no convulsions.

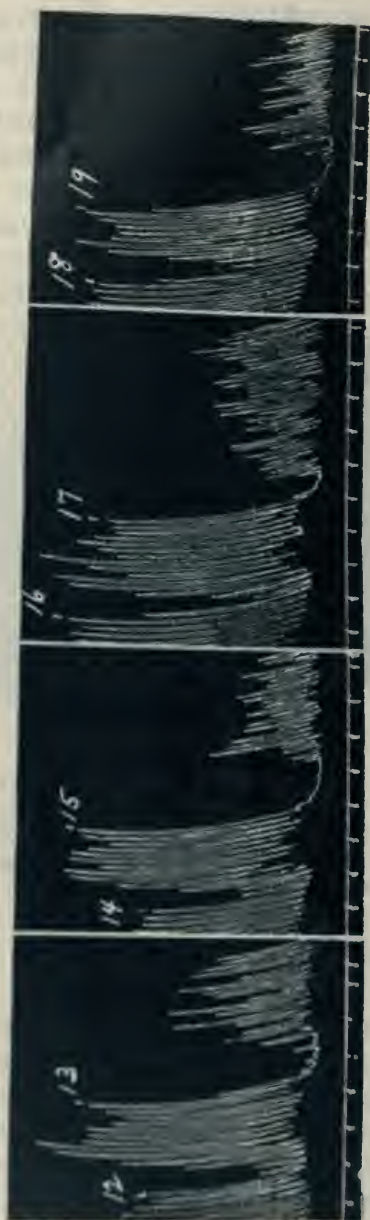


FIG. 6. INTESTINE TRACINGS. BLOODS FROM DOG 257

At 12, 14, 16 and 18 Ringer was replaced by indifferent (jugular) blood, and this at 13 by jugular blood made up with adrenalin to a concentration of 1:2,500,000; at 15 by the eighth adrenal blood specimen (collected forty-five minutes after strychnine); at 17 by jugular blood made up with adrenalin to a concentration of 1:1,660,000; and at 19 by jugular blood made up with adrenalin to a concentration of 1:830,000. All the bloods were diluted with three volumes Ringer, the adrenal in bloods after addition of the adrenalin. (Reduced to three-fifths.)

The fourth and sixth specimens did not show any definite increase in the output of epinephrin as compared with the second specimen. It was apparently after the second dose of strychnine, that the output was markedly increased.

In the next experiment (dog 246) a very large dose of strychnine was administered, causing convulsions almost immediately, and the epinephrin output was markedly increased in the specimen collected two minutes thereafter.

Condensed protocol; dog 246; female; weight, 7.5 kgm.

Anesthetized with morphine and ether. Obtained a specimen of indifferant blood from external jugular vein. Made cava pocket.

11.35 a.m. Started artificial respiration.

11.40 a.m. Blood pressure 110 mm. Hg. Collected adrenal blood. First specimen, 8.8 grams in 30 seconds (17.6 grams per minute). Second specimen, 7.7 grams in 30 seconds (15.4 grams per minute).

11.52 a.m. Injected 2 mgm. strychnine into jugular vein. Powerful tetanic convulsions ensued almost immediately, and persisted throughout the collection of third and fourth specimens.

11.54 a.m. Third specimen, 7.3 grams in 30 seconds (14.6 grams per minute). Fourth specimen, 8.3 grams in 30 seconds (16.6 grams per minute).

12.00 m. Blood pressure 170 mm. Hg.

12.25 p.m. Tonic and clonic convulsions continued since collection of last adrenal specimen and dog died in convulsions at 12.28.

Combined weight of adrenals 1.26 grams.

The fourth specimen (collected after the strychnine injection) produced an enormously greater inhibition of the intestine segment than the second specimen (collected before strychnine was given) (fig. 7, observations 3 and 5). As the blood flow during collection of the fourth was somewhat greater than during collection of the second, the mere comparison of the curves is sufficient to show that the epinephrin output was increased. The assay proved that the second specimen was weaker than

1:10,000,000 adrenalin, weaker than 1:12,500,000, approximately the same as 1:15,000,000 (confirmed by several pairs of observations). Taking the second specimen at 1:15,000,000, we get 0.001 mgm. per minute for the dog, or 0.00013 mgm. per kilogram per minute. The fourth specimen was shown to be much stronger than 1:3,750,000 adrenalin, stronger than 1:2,500,000, stronger than 1:1,875,000; not very different from 1:1,250,000, probably somewhat weaker. Taking the fourth specimen even at 1:1,500,000, we get 0.011 mgm. per minute for the dog, or 0.0015

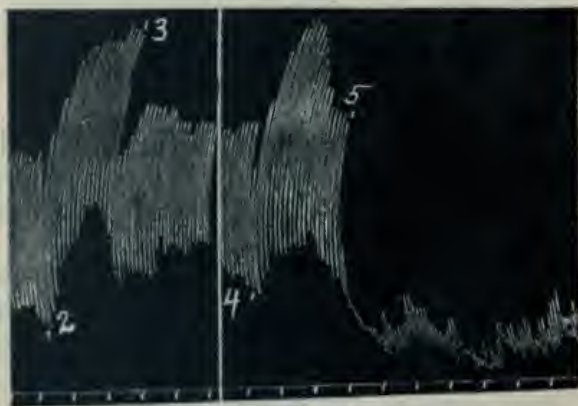


FIG. 7. INTESTINE TRACINGS. BLOODS FROM DOG 246

At 2 and 4 Ringer was replaced by indifferent (jugular) blood, and this at 3 by the second adrenal specimen (collected before strychnine); at 5 by the fourth adrenal specimen (collected three minutes after strychnine). The bloods were diluted with three volumes Ringer. (Reduced to one-half.)

mgm. per kilogram per minute. This is more than ten times the initial output.

It had been intended to wait for a considerable time before collecting a further specimen of adrenal blood, in order to see whether the increase in the epinephrin output produced by strychnine was transient or lasting. The animal died, however, in convulsions thirty minutes after the administration of the strychnine. No exact correspondence between the dose of strychnine and the increase in the epinephrin output could of course be expected. Something depends upon the initial rate

of output. If this is large it is scarcely likely that the relative increase produced by a given dose of strychnine will be as great as when the initial output is relatively low. There are, of course, other factors, among which the degree and nature of the anesthesia must be important, which influence the result. Thus, the largest dose given to dogs in the whole series of experiments was in dog 245. The dog died eventually in severe convulsions. The output of epinephrin in a specimen of adrenal blood collected four minutes after the injection of the strychnine was not quite twice as great as the relatively high output before the administration of the strychnine, in spite of the extreme excitation of the spinal motor mechanisms.

Condensed protocol; dog 245; male; weight, 9.5 kgm.

Anesthetized with morphine and ether. Obtained a specimen of indifferent blood from external jugular vein. Made cava pocket. Blood pressure 70 mm. Hg. Collected adrenal blood.

- 11.50 a.m. First specimen, 5.4 grams in 30 seconds (10.8 grams per minute). Second specimen, 9.5 grams in 60 seconds (9.5 grams per minute). Third specimen, 10 grams in 60 seconds (10 grams per minute). Started artificial respiration. Blood pressure 68 mm. Hg.
- 12.15 p.m. Injected 4.0 mgm. strychnine into jugular; tetanic convulsions occurred at once.
- 12.19 p.m. Fourth specimen, 4.25 grams in 16 seconds (16 grams per minute). Fifth specimen, 12.15 grams in 60 seconds (12.15 grams per minute).
- 12.25 p.m. Blood pressure 84 mm. Hg.
- 12.45 p.m. Dog died in severe convulsions.

Combined weight of adrenals 1.55 grams.

The third specimen (collected before strychnine) assayed at 1:3,500,000, the fifth (four minutes after strychnine) at 1:2,500,000. Figure 8 is reproduced to emphasize the point that while mere comparison of the inhibitory effects produced on the intestine segment by these two specimens (observation 3 and 5) is sufficient to show that the output of epinephrin must

have been increased in the fifth specimen, since the blood flow was somewhat greater in the fifth than in the third, no quantitative estimate of the difference can be made without a careful and detailed assay. The difference in concentration between the specimens was in reality much less than casual comparison of the curves might suggest.

In the next experiment (dog 248) a smaller, but still a large dose in proportion to the bodyweight was given, and specimens of adrenal blood were collected immediately (two and one-half minutes) after administration of the drug and forty minutes thereafter.

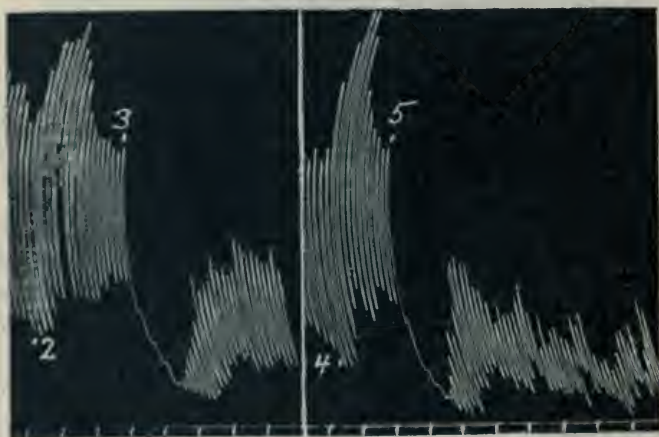


FIG. 8. INTESTINE TRACINGS. BLOODS FROM DOG 245

At 2 and 4 Ringer was replaced by indifferent (jugular) blood, and this at 3 by the third adrenal blood specimen (collected before strychnine); at 5 by the fifth adrenal specimen (collected five minutes after strychnine). The bloods were diluted with three volumes Ringer. (Reduced to one-half.)

Condensed protocol; dog 248,; female; weight, 4.6 kgm.

Anesthetized with morphine and ether. Obtained a specimen of indifferent blood from the external jugular vein. Made cava pocket. Then collected adrenal blood. Blood pressure before collection of first adrenal specimen 58 mm. Hg.

12.00 m. First specimen, 5 grams in 30 seconds (10 grams per minute). Second specimen, 9.7 grams in 60 seconds (9.7 grams per minute).

- 12.15 p.m. Injected 0.5 mgm. strychnine into jugular vein. Severe tetanic convulsions occurred within one-half minute and artificial respiration was started at once.
- 12.17 p.m. Third specimen, 6.5 grams in 40 seconds (9.6 grams per minute). Fourth specimen, 8.9 grams in 60 seconds (8.9 grams per minute).
- 12.20 p.m. Blood pressure after collection of fourth specimen 125 mm. Hg.
- 12.26 p.m. Reflexes increased; convulsions ceased; spontaneous breathing.
- 12.40 p.m. Pulse 190 to 200 per minute; reflexes increased; spontaneous breathing.
- 12.50 p.m. Stopped artificial respiration.
- 12.55 p.m. Fifth specimen, 5.1 grams in 35 seconds (9.2 grams per minute). Sixth specimen, 8.15 grams in 60 seconds (8.15 grams per minute). Blood pressure after collection of sixth adrenal specimen 70 mm. Hg.

Obtained more jugular blood, and also arterial blood (from abdominal aorta). Combined weight of adrenals 0.8 gram.

The second specimen (taken before strychnine), the fourth (taken two and one-half minutes after strychnine) and the sixth (taken forty minutes after the administration of the drug) were assayed. As the blood flow differed little in the three specimens, even a comparison of their effects on the intestine segment (fig. 9) indicates that the output must have been greatly increased in the case of the fourth, and increased, although not so much in the case of the sixth specimen.

The assay showed that the second specimen was weaker than 1:10,000,000 adrenalin, weaker than 1:12,500,000, somewhat weaker than 1:15,000,000, and stronger than 1:18,750,000. It was confirmed by several observations that the concentration of the second specimen was less than 1:15,000,000 and probably not much greater than 1:18,750,000. Even if we take it at 1:16,000,000, we get for the epinephrin output before strychnine only 0.0006 mgm. per minute for the dog, or 0.00013 mgm. per kilogram per minute.

While the second specimen was assayed soon after the blood was collected, the fourth and sixth could only be provisionally

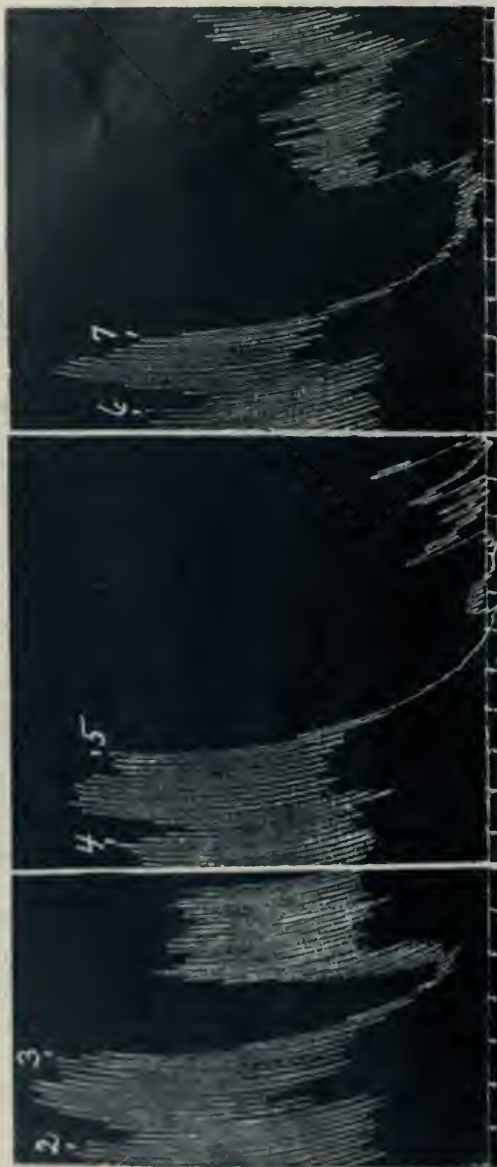


FIG. 9. INTESTINE TRACINGS. BLOODS FROM DOG 248

At 2, 4, and 6 Ringer was replaced by indifferent (jugular) blood, and this at 3 by the second adrenal specimen (collected before strychnine); at 5 by the fourth adrenal specimen (collected one minute after strychnine); at 7 by the sixth adrenal specimen (collected forty minutes after strychnine). The bloods were diluted with three volumes Ringer. (Reduced to three-fifths.)

assayed at that time. The assay was not completed till next day, the bloods being meanwhile kept on ice. Some epinephrin must, therefore, have been lost by these specimens, and the increase in the output is even greater than that yielded by the assay. The fourth specimen was much stronger than 1:3,750,000, stronger than 1:2,500,000 (confirmed by several observations), approximately the same as 1:1,250,000.

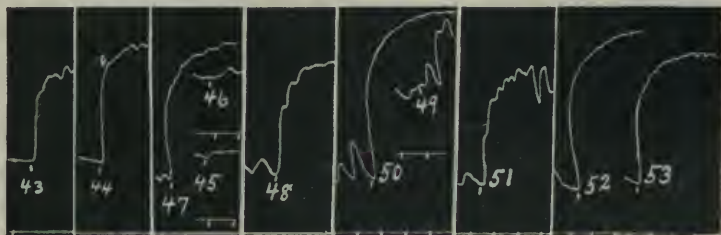


FIG. 10. UTERUS TRACINGS. BLOODS FROM DOG 248

At 43 Ringer was replaced by the sixth adrenal specimen (collected forty minutes after strychnine); at 44 by the fourth adrenal specimen (collected one minute after strychnine); at 45 by the second adrenal specimen (collected before strychnine); at 46 by arterial blood (collected after strychnine); at 47 by arterial blood made up with adrenalin to a concentration of 1:2,500,000; at 48 by arterial blood made up with adrenalin to a concentration of 1:3,750,000; at 49 by arterial blood made up with adrenalin to a concentration of 1:18,750,000; at 50 by the fourth adrenal specimen; at 51 by arterial blood made up with adrenalin to a concentration of 1:3,150,000; at 52 by the sixth adrenal blood specimen; and at 53 by arterial blood made up with adrenalin to a concentration of 1:3,150,000. All the bloods were diluted with one volume of Ringer, the adrenalin bloods after addition of the adrenalin. (Reduced to two-fifths.)

Taking the fourth specimen at 1:1,250,000, we get 0.007 mgm. per minute for the dog, or 0.0015 mgm. per kilogram per minute, fully ten times the initial output.

The sixth specimen was found to be much stronger than 1:5,000,000 adrenalin by intestine observations, and stronger than 1:3,125,000 (by uterus observations made next day, figure 10, observations 52 and 53, confirmed by observation 51). It was not as exactly assayed as the second and fourth specimens, but its concentration even twenty-four hours after collection indicated that the rate of output of epinephrin must have been

at least seven or eight times the initial output forty minutes after the administration of the strychnine. The conclusions drawn from the intestine assay were confirmed also for the other specimens by uterus observations (fig. 10).

The next experiment (dog 263) is an example of observations in which with a relatively small dose of strychnine, which caused only an increased reflex excitability unaccompanied at any time by convulsions, no increase in the output of epinephrin may be produced in the first specimen of adrenal blood drawn after the administration of the strychnine, but in later specimens a gradually increasing rate of output can be demonstrated; the increased output lasting for a considerable time, even when the exaggerated reflex excitability has disappeared.

Condensed protocol; dog 263; male; weight, 4.4 kgm.

Anesthetized with morphine and ether. Obtained a specimen of indifferent blood from femoral vein. Made cava pocket. Blood pressure 94 mm. Hg. Collected adrenal blood.

- 12.10 p.m. First specimen, 3.3 grams in 30 seconds (6.6 grams per minute). Second specimen, 6.15 grams in 69 seconds (6.15 grams per minute).
- 12.25 p.m. Injected 0.25 mgm. strychnine into jugular vein. Blood pressure 92 mm. Hg.—exaggerated reflexes.
- 12.26½ p.m. Third specimen, 3.35 grams in 30 seconds (6.7 grams per minute).
- 12.27 p.m. Fourth specimen, 5.7 grams in 60 seconds (5.7 grams per minute).
- 12.45 p.m. Blood pressure 88 mm. Hg. Fifth specimen, 3.5 grams in 30 seconds (7 grams per minute). Sixth specimen, 5.75 grams in 60 seconds (5.75 grams per minute).
- 12.50 p.m. Closed abdomen with clamps.
- 1.45 p.m. Blood pressure 76 mm. Hg.
- 1.49½ p.m. Seventh specimen, 2.2 grams in 30 seconds (4.4 grams per minute).
- 1.50 p.m. Eighth specimen, 7.4 grams in 120 seconds (3.7 grams per minute).

Obtained arterial blood from abdominal aorta. Combined weight of adrenals 0.78 gram.

In this animal the initial output before the strychnine injection was rather high. The second specimen was shown to be stronger than 1:4,300,000 adrenalin; decidedly weaker than 1:2,500,000 and a little weaker than 1:3,500,000. It was finally taken as equal to 1:3,800,000, giving 0.0016 mgm. per minute for the animal, or 0.00035 mgm. per kilogram per minute. The fourth specimen (collected two minutes after the strychnine injection) was somewhat weaker than the second, although the blood flow was rather less. It was assayed at 1:4,300,000, corresponding to an output per minute for the dog of 0.0013 mgm., or 0.0003 mgm. per kilogram per minute. We cannot always be certain of a difference as small as this, but in this case there seems no doubt from the sharpness of the assay, and from the fact that qualitatively the fourth specimen gave a smaller reaction than the second, in spite of the smaller blood flow during collection of the former, that the output was somewhat diminished in the first stage of the strychnine action. If this were an isolated observation we should not think of basing such a conclusion upon it. But we have seen in other experiments much more striking instances of a transient preliminary diminution in the epinephrin output after the administration of strychnine. It is to be expected that if such a phenomenon exists, it will be more easy to detect it when small doses of the drug are used, or where it is administered subcutaneously instead of intravenously and where accordingly the absorption is slower, and the transient inhibitory action of the drug is not masked by a rapidly developed, large and lasting augmentation of the rate of output. It is precisely in these circumstances that the evidence of the preliminary diminution in the output is clearest. Instances of this will be given in the proper place.

The sixth specimen (collected ten and one-half minutes after the strychnine injection) showed a definite augmentation of the epinephrin output. It was assayed at 1:2,800,000, corresponding to an output for the dog of 0.002 mgm. per minute, or 0.00045 mgm. per kilogram per minute. The eighth specimen (obtained eighty-five minutes after administration of the strychnine) was found to be stronger than 1:1,400,000 adrenalin,

slightly weaker than 1:1,000,000. It was taken as equal to 1:1,100,000, corresponding to an output of 0.0034 mgm. per minute for the animal, or 0.00075 mgm. per kilogram per minute, fully double the initial output. The percentage of serum in the eighth specimen was 66.5 determined by the electrical method, and 65 per cent by the haematocrit with prolonged centrifugalisation. The concentration of epinephrin in the serum was therefore 1:730,000.

Having demonstrated in the experiments on dogs, with intravenous injection of strychnine, the effects produced on the rate of epinephrin output by large and small doses, some experiments were next made on the effects of the drug when administered subcutaneously. We began with a large (convulsant) dose in order to see at once whether the general course of the action was the same.

This proved to be the case, the most notable point of difference being the clearer indication of a transient preliminary stage of inhibition or reduction in the rate of liberation of epinephrin.

Condensed protocol; dog 306; male; weight, 5.05 kgm.

Anesthetized with morphine and ether. Obtained indifferent blood from the jugular vein. Made cava pocket. Then collected adrenal blood.

- 11.30½ a.m. First specimen, 3.6 grams in 30 seconds (7.2 grams per minute).
- 11.31 a.m. Second specimen, 8.2 grams in 60 seconds (8.2 grams per minute). Blood pressure 84 mm. Hg.
- 11.37½ a.m. Injected 1.0 mgm. strychnine hypodermically.
- 11.40 a.m. Injected 1.0 mgm. strychnine hypodermically.
- 11.44 a.m. Tonic convulsions—started artificial respiration, which was continued for the rest of the experiment.
- 11.44½ a.m. Third specimen, 4 grams in 30 seconds (8 grams per minute).
- 11.45 a.m. Fourth specimen, 7.8 grams in 60 seconds (7.8 grams per minute). Blood pressure 115 mm. Hg.
- 11.50 a.m. Fifth specimen, 16.1 grams in 60 seconds (16.1 grams per minute). Blood pressure 110 mm. Hg.

- 11.58 a.m. Clonic convulsions—spontaneous breathing.
12.09 p.m. Sixth specimen, 6.3 grams in 30 seconds (12.6 grams per minute).
12.09½ p.m. Seventh specimen, 11.1 grams in 60 seconds (11.1 grams per minute).
12.10½ p.m. Eighth specimen, 9.55 grams in 60 seconds (9.55 grams per minute). Blood pressure 60 mm. Hg.
12.30 p.m. Ninth specimen, 3.35 grams in 30 seconds (6.7 grams per minute).
12.30½ p.m. Tenth specimen, 7.55 grams in 60 seconds (7.55 grams per minute). Clonic convulsions still present; spontaneous breathing. Blood pressure 50 mm. Hg.

Abdominal aorta blood obtained. Combined weight of adrenals 0.705 gram.

The maximum increase was as large as in any of the experiments with intravenous injection (up to ten times the initial output). More than half an hour after injection of the strychnine the output was still eight times as great as before the drug was administered. Fifty minutes after the injection the output was still distinctly increased. Specimens of the tracings are given in figures 11 to 15. In figure 11 it is demonstrated that the fourth adrenal specimen, collected five minutes after the last injection of strychnine, while strong tonic convulsions were going on, caused no inhibition of the intestine segment, while the second specimen, collected before injection of strychnine caused a distinct effect. The difference was considerably greater in other observations when the segment had become more sensitive. Since the blood flows during collection of the second and fourth specimens were practically equal, a little less indeed, for the fourth, this is of itself sufficient to show that the output at the time of collection of the fourth specimen was diminished. Comparison of the effect produced by the fourth with that produced by the fifth specimen (fig. 12) indicates a very great increase in the output in the five minutes following the collection of the fourth specimen.

The epinephrin assay showed that the second specimen (collected before strychnine was given) was decidedly weaker than 1:5,000,000 adrenalin, weaker than 1:6,500,000, distinctly

weaker than 1:7,000,000, and somewhat weaker than 1:7,850,000 (confirmed by three separate pairs of observations). It was stronger than 1:10,000,000, and approximately equal to 1:8,500,000 (fig. 13), corresponding to an output of 0.001 mgm. per minute for the dog, or 0.0002 mgm. per kilogram per minute.

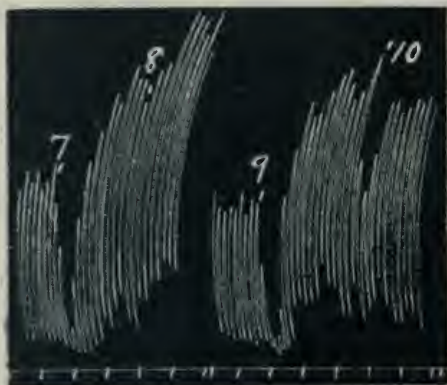


FIG. 11. INTESTINE TRACINGS. BLOODS FROM DOG 306

At 7 and 9 Ringer was replaced by arterial blood, and this at 8 by the fourth adrenal specimen; at 10 by arterial blood made up with adrenalin to a concentration of 1:15,000,000. The bloods were diluted with one volume Ringer, the adrenalin blood after addition of the adrenalin. (Reduced to one-half.)

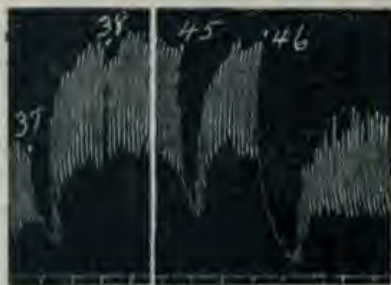


FIG. 12. INTESTINE TRACINGS. BLOODS FROM DOG 306

At 37 and 45 Ringer was replaced by indifferent (arterial) blood (collected after strychnine); at 38 by the fourth adrenal specimen (collected eight minutes after injection of strychnine) at 48 by the fifth adrenal specimen (collected ten minutes after strychnine). The bloods were diluted with three volumes Ringer. (Reduced to one-half.)

It was shown that 1:30,000,000 adrenalin gave a distinct inhibition at a time when the fourth specimen caused practically no effect. The fourth specimen must, therefore, have been weaker than 1:30,000,000. It was not proved that it contained

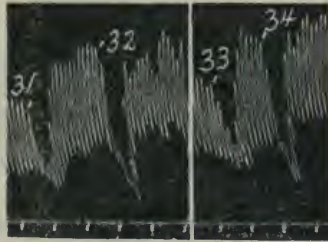


FIG. 13. INTESTINE TRACINGS. BLOODS FROM DOG 306

At 31 and 33 Ringer was replaced by indifferent (jugular) blood, and this at 32 by jugular blood with adrenalin added to make a concentration of 1:8,500,000; at 34 by the second adrenal specimen (collected before strychnine). The bloods were diluted with three volumes Ringer, the adrenalin blood after adding the adrenalin. (Reduced to one-half.)

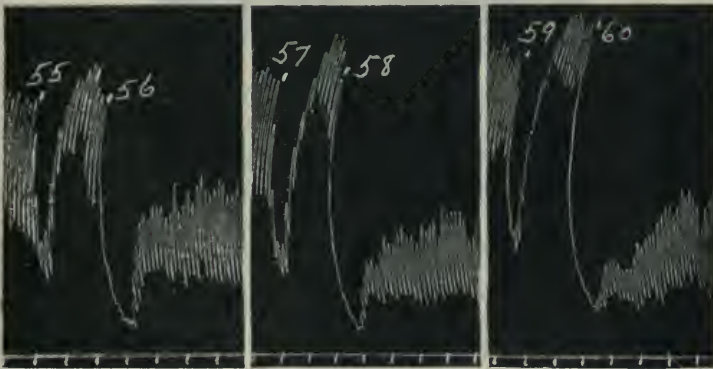


FIG. 14. INTESTINE TRACINGS. BLOODS FROM DOG 306

At 55, 57 and 59 Ringer was replaced by arterial blood (collected after strychnine); at 58 the eighth adrenal specimen (collected thirty minutes after subcutaneous injection of strychnine) replaced the arterial blood; at 56 and 60 the arterial blood was replaced by arterial blood made up with adrenalin to a concentration of 1:1,400,000 and 1:1,000,000 respectively. All the bloods were diluted with three volumes Ringer, the adrenalin bloods after addition of the adrenalin. (Reduced to one-half.)

any epinephrin. The output at the time of collection of this specimen was certainly less than 0.00025 mgm. per minute for the animal, or 0.00005 mgm. per kilogram per minute, i.e., less than one-fourth the initial output before administration of strychnine. The reduction was probably considerably greater than this.

The fifth specimen, collected ten minutes after the strychnine injection, in spite of the greatly increased blood flow had a much greater concentration of epinephrin than the second. It was found to be stronger than 1:2,250,000 adrenalin, somewhat weaker than 1:1,500,000. It was taken at 1:1,600,000, corresponding to an output of 0.01 mgm. per minute for the dog,



FIG. 15. UTERUS TRACINGS. BLOODS FROM DOG 306

At 58, Ringer was replaced by jugular blood; at 59 by the tenth specimen; at 60 by the fifth specimen; at 61 by the eighth specimen. All the bloods were diluted with five volumes Ringer. (Reduced to one-half.)

or 0.002 mgm. per kilogram per minute; ten times the initial output.

The eighth specimen, collected half an hour after injection, was found to be decidedly stronger than 1:3,000,000 adrenalin, stronger than 1:1,400,000, probably not quite as strong as 1:1,000,000, but not much different from it (fig. 14). It was taken at 1:1,200,000, corresponding to an output of 0.008 mgm. per minute for the dog, or 0.0016 mgm. per kilogram per minute; eight times the initial output. The proportion of serum in the blood was 46.6 per cent as determined by the electrical method. The haematocrit, even after thirty-two and one-half minutes, gave only 33 per cent, although the proportion was slowly mount-

ing as the centrifugalization went on. The haematocrit method is quite unsuitable when the proportion of serum is small and the corpuscles separate slowly. The concentration of epinephrin in the serum of the eighth specimen, taking the percentage at 46.5, was 1:560,000, very nearly the possible normal maximum. Since this maximum does not seem to be altered by strychnine, although as will be shown in another paper it may be markedly affected by other drugs, the calculated output for the eighth specimen might have been even greater had there been at this time a larger blood flow to carry off the epinephrin.

The tenth specimen, collected fifty minutes after the administration of strychnine, could not be satisfactorily assayed owing to a mistake, but it was shown that its concentration was such as to leave no doubt that at this time the epinephrin output was still decidedly increased.

The uterus tracings reproduced in figure 15 confirm the intestine observations and show that the inhibitory reactions of the intestine segments were due to epinephrin. The fourth specimen gave with the uterus a very small reaction compared with the other specimens.

In the next experiment, the last on dogs to be cited, a much smaller quantity of strychnine (two doses amounting to 0.09 mgm. per kilogram of bodyweight in all) injected subcutaneously was found to cause a distinctly increased output of epinephrin. The dose was below that required to produce any noticeable effect on the reflex excitability. The blood pressure was not altered nor would it have been possible for anyone studying the behavior of the animal to conclude that strychnine had been administered.

Condensed protocol; dog 309; female; weight, 8.1 kgm.

Anesthetized with morphine and ether. Obtained a specimen of indifferent blood from the jugular vein. Made cava pocket. Then collected adrenal blood.

11.00 a.m. Blood pressure 108 mm. Hg.

11.01½ a.m. First specimen, 7.25 grams in 30 seconds (14.5 grams per minute).

- 11.02 a.m. Second specimen, 15.9 grams in 60 seconds (15.9 grams per minute).
11.09 a.m. Injected 0.5 mgm. strychnine hypodermically.
11.12 a.m. Reflexes not increased.
11.15 a.m. Injected 0.25 mgm. strychnine hypodermically.
11.19 a.m. No noticeable increase of reflexes.
11.21 a.m. Third specimen, 6.8 grams in 30 seconds (13.6 grams per minute).
11.21½ a.m. Fourth specimen, 13.6 grams in 60 seconds (13.6 grams per minute).
11.22½ a.m. Blood pressure 100 mm. Hg.
11.25 a.m. Reflexes not demonstrably increased; administered a few whiffs of ether.
11.37 a.m. Fifth specimen, 5.2 grams in 30 seconds (10.4 grams per minute).
11.37½ a.m. Sixth specimen, 11.1 grams in 60 seconds (11.1 grams per minute).
11.38½ a.m. Blood pressure 88 mm. Hg.
12.07 p.m. Seventh specimen, 4.2 grams in 30 seconds (8.4 grams per minute).
12.07½ p.m. Eighth specimen, 10.4 grams in 60 seconds (10.4 grams per minute).
12.08½ p.m. Blood pressure 85 mm. Hg.

No distinctly increased reflex excitability was present at any time. Another specimen of indifferent blood was obtained from the right heart while the animal was still in good condition. Combined weight of adrenals 0.942 gram.

The epinephrin assay showed that the second adrenal specimen (collected before strychnine) was stronger than the fourth specimen (collected six and one-half minutes after the last dose of strychnine), although the blood flow was somewhat greater for the second than for the fourth (fig. 16, observations 2 and 4). This is a further indication that a transient diminution of the output of epinephrin may precede the increase, although, of course, if this was an isolated observation no weight would be attached to such a relatively small difference.

The sixth adrenal specimen (taken more than twenty-two minutes after the last dose of strychnine) was distinctly stronger

than the second (fig. 16, observation 6). The assay showed that the second specimen was much stronger than 1:10,000,000 adrenalin, weaker than 1:5,000,000 (confirmed by several observations), stronger than 1:7,100,000, and approximately the same as 1:6,100,000, corresponding to an output of 0.0026 mgm. per minute for the dog, or 0.0003 mgm. per kilogram per minute.

The sixth specimen was found to be stronger than 1:3,500,000, stronger than 1:3,000,000 (confirmed by several observations), somewhat weaker than 1:2,100,000 (fig. 17, observations 60 and 62, confirmed by other observations). It was finally taken at

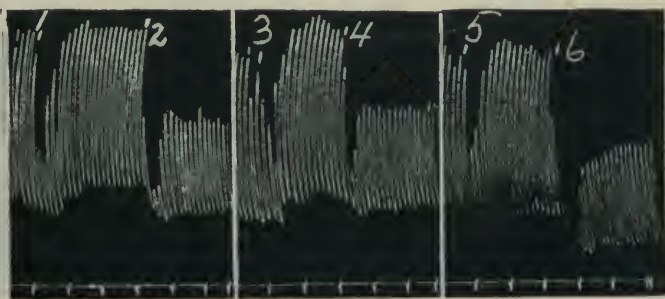


FIG. 16. INTESTINE TRACINGS. BLOODS FROM DOG 309

At 1, 3 and 5 Ringer was replaced by jugular blood, and this at 2 by the second adrenal specimen (collected before injection of strychnine); at 4 by the fourth adrenal specimen (collected six minutes after subcutaneous injection of strychnine), at 6 by the sixth adrenal specimen (collected twenty-five minutes after injection of strychnine). All the bloods were diluted with three volumes Ringer. (Reduced to one-half.)

1:2,300,000, corresponding to an output of 0.0048 mgm. per minute for the dog, or 0.0006 mgm. per kilogram per minute. At this stage, therefore, the initial output was doubled.

The eighth specimen (collected about an hour after the first dose of strychnine) was shown to be much stronger than 1:3,000,000, much stronger than 1:2,500,000, stronger than 1:2,100,000 (fig. 17, observations 62 and 64), and weaker than 1:1,800,000 (fig. 17, observations 64 and 66). It was confirmed by the uterus that the eighth was the strongest of all the specimens. It was finally taken at 1:2,000,000, corresponding to an output of 0.005 mgm. per minute for the dog, or 0.0006 mgm.

per kilogram per minute. The increase in the output was at least as great as half an hour earlier. It is not known whether the crest of the increase might not have been reached somewhere between the sixth and eighth specimens, in which case the maximum increase would have been greater than that found in either specimen.

Corroborative evidence that strychnine increases the output of epinephrin was obtained by studying the effect upon the blood pressure of adrenal vein blood collected in a cava pocket for a definite time, before and after administration of strychnine and

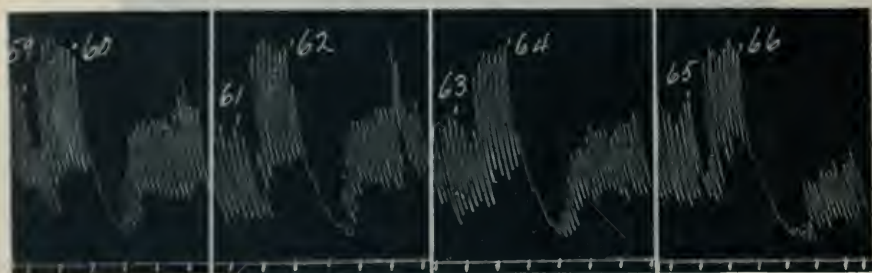


FIG. 17. INTESTINE TRACINGS. BLOODS FROM DOG 309

At 59, 61, 63, and 65 Ringer was replaced by jugular blood, and this at 60 by the sixth adrenal specimen (collected twenty-five minutes after injection of strychnine); at 62 by jugular blood to which was added adrenalin to make a concentration of 1:2,100,000; at 64 by the eighth adrenal specimen (collected fifty minutes after injection of strychnine); at 66 by jugular blood to which was added adrenalin to make a concentration of 1:1,800,000. All the bloods were diluted with three volumes Ringer, the adrenalin bloods after adding the adrenalin. (Reduced to one-half.)

then released into the circulation. It is not easy, with large doses, to overcome the difficulty caused by the great rise of blood pressure produced by the strychnine itself and which is not eliminated by curare. But we succeeded in getting a sufficient number of satisfactory tracings to demonstrate by this method also that strychnine produces a definite increase in epinephrin output. One experiment (on dog 278) is cited as an example, with specimens of the blood pressure tracings (figs. 18 to 21). The animal, a bitch weighing 5.6 kilograms, was anesthetized with morphine

and ether. A "long" cava pocket was formed in the usual way, the abdominal aorta, but not the intestinal arteries being tied. A blood pressure tracing was taken from a carotid. The vagi were cut. Numerous pocket experiments were made to determine the output of epinephrin and the effect of strychnine upon it. In figure 18, before the administration of strychnine, the pocket was closed at 2, opened after ninety seconds at 3. The mean arterial pressure before the opening of the pocket was 84 mm. of mercury, the maximum after opening 106 mm. 0.5 mgm. of strychnine sulphate was injected into the jugular two to three minutes before 12 (fig. 18) in two doses. Convulsions ensued, but

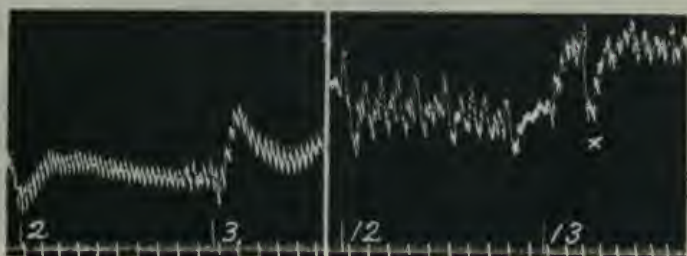


FIG. 18. BLOOD PRESSURE TRACINGS FROM DOG 278

Cava pocket closed at 2, opened after ninety seconds at 3 (before strychnine); cava pocket closed at 12, opened after ninety seconds at 13 (four minutes after strychnine injection). The abrupt descent in the curve marked by a cross coincided with a convulsion. The signal marks on the time trace are 2 mm. to the right of the corresponding points on the blood pressure curve. Time in ten-second intervals. Line of zero pressure (same as time trace) moved up 27 mm. and the figure then reduced to two-thirds.

the natural breathing continued to be sufficient. At 12 (four minutes after the first dose of strychnine) the cava pocket was closed, at 13 it was opened after ninety seconds. The mean pressure before the opening of the pocket was 105 mm. of mercury and the maximum pressure after it was released 140 mm. Obviously the adrenal blood collected in the pocket after strychnine, caused a decidedly greater effect on the blood pressure than the blood collected for the same time before strychnine. Not only was the rise of pressure after strychnine greater, but it was much more sustained, the maximum level being maintained for some

minutes after 13. An approximate estimate of the rate of output of epinephrin before and after strychnine (fig. 19) showed also a definite increase after strychnine. The pocket was closed at 5 (before strychnine), and opened at 7 after two minutes. The effect produced on the blood pressure at 7 is manifestly less than that due to injection into the jugular at 6 of 0.5 cc. of a 1:300,000 solution of adrenalin. Nine minutes after the strychnine injection a corresponding pocket experiment (14 to 16) was made. Although the time of closure of the pocket was only ninety seconds, the rise of pressure following release of the pocket was

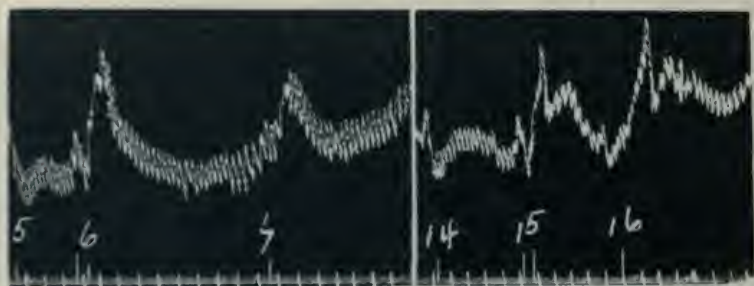


FIG. 19. BLOOD PRESSURE TRACINGS FROM DOG 278

Cava pocket closed at 5 (before strychnine injection), opened after two minutes at 7. At 6, 0.5 cc. of 1:300,000 adrenalin injected into jugular. Cava pocket (nine minutes after strychnine injection) closed at 14, opened after ninety seconds at 16. At 15, 0.5 cc. of 1:300,000 adrenalin injected into jugular. The signal marks on the time trace are 2 mm. to the right of the corresponding points on the blood pressure curve, Line of zero pressure (same as time trace) moved up 33 mm. and the figure then reduced to two-thirds.

distinctly greater and more sustained than that due to injection of 0.5 cc. of 1:300,000 adrenalin.

In both cases the adrenalin was injected while the pocket was closed, as we have seen some evidence that the effect of a given quantity of adrenalin injected into the circulation, may not be quite the same when the normal epinephrin discharge into the blood is going on, as when the adrenal blood is prevented from escaping. Any evidence that a given amount of adrenalin affects the blood pressure differently according to whether the normal epinephrin discharge is occurring or not is at the same time evi-

dence that this normal discharge is exerting some influence upon the circulation. While we have not made any special experiments on this point and have only incidental observations to go upon, it is obvious that if the normal amount of epinephrin continuously liberated from the adrenals is already affecting the circulatory mechanism to a sensible extent, the change produced by the addition of a given amount of adrenalin may be less than when the same amount of adrenalin is thrown in at a time when epinephrin has disappeared from the blood stream. In the one case the increase in circulation will be from zero, in the other from a small, but still positive amount. This factor would naturally be still more important when the epinephrin output under the influence of strychnine was proceeding at an accelerated rate. Be this as it may, it is clear that the conditions are more nearly similar when we compare the action of a given dose of adrenalin injected while the pocket is closed off and, therefore, into a circulation bare of epinephrin with the effect of adrenal blood released from the pocket, than when the adrenalin is injected with the normal epinephrin discharge proceeding. It will be seen from the curves that there is plenty of time with the duration of pockets employed, for the adrenalin reaction to have passed off before the pocket is released. Complete equality in the conditions could be attained by clamping off the pocket after it was emptied, so as to ensure that the blood pressure reaction caused by the contents of the pocket is not reinforced by the succeeding steady discharge.

The epinephrin output before administration of strychnine, was approximately estimated by auto-assay at 0.0006 mgm. per minute for the dog, or 0.0001 mgm. per kilogram per minute. Ten minutes after injection of strychnine an approximate assay gave 0.0013 mgm. per minute for the dog, or 0.00023 mgm. per kilogram per minute. The assays were rather rough as it was necessary to estimate rapidly the increasing output as the strychnine action developed, and at best, blood pressure assays cannot be as delicate as those made with rabbit segments. Nevertheless, it is certain that the output of epinephrin was at least doubled in the first ten minutes after the strychnine was given.

Thirty minutes after injection of strychnine the rise of pressure produced by releasing a pocket which had been closed for ninety seconds was much greater than that caused by injection of 0.5 cc. of 1:150,000 adrenalin and decidedly less than that caused by 0.5 cc. of 1:75,000 adrenalin (fig. 20).

The output at this time was at least 0.0025 mgm. per minute for the dog, or 0.00045 mgm. per kilogram per minute; fully four times the output before strychnine. The adrenalin injections at this point were made with the pocket open. Several additional pocket experiments and injections of adrenalin with the pocket open and closed confirmed the conclusion that strychnine

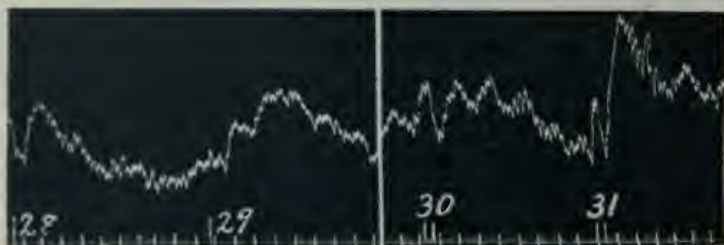


FIG. 20. BLOOD PRESSURE TRACINGS FROM DOG 278

Cava pocket (thirty-four minutes after strychnine injection) closed at 28, opened after ninety seconds at 29. At 30, 0.5 cc. of 1:150,000 adrenalin; at 31, 0.5 cc. of 1:75,000 adrenalin injected into jugular. Time in ten second intervals. The signal marks on the time trace are 2 mm. to the right of the corresponding points on the blood pressure curve. Line of zero pressure (same as time trace) moved up 27 mm. and the figure then reduced to two-thirds.

had caused a decided increase in the rate of epinephrin output and that the increase was greater forty to fifty minutes after the strychnine injection than ten to fifteen minutes after it. Curare (2 cc. of a 1 per cent solution) had been administered intravenously in the interval between the tracings shown in figures 19 and 20. Paralysis was complete in ten to fifteen minutes and artificial respiration was maintained thereafter. That some of the bulbo-spinal centres were still extremely sensitive to strychnine was shown by the effect of the intravenous injection of 0.5 mgm. forty minutes after the administration of curare and fourteen minutes after the last observation reproduced in figure 20.

Twenty seconds after injection of the strychnine the blood pressure rose almost vertically from 100 mm. to 230 mm. of mercury. The rise was not accompanied by any muscular contraction and was long maintained. After twelve minutes the pressure was still 200 mm. Preparations were now made to expose the cervical cord. There was sharp hemorrhage and the pressure fell to 110 mm. before the canal was opened. On sectioning the cord the pressure fell to 80 mm., then to 40 mm. and finally to 10 mm.

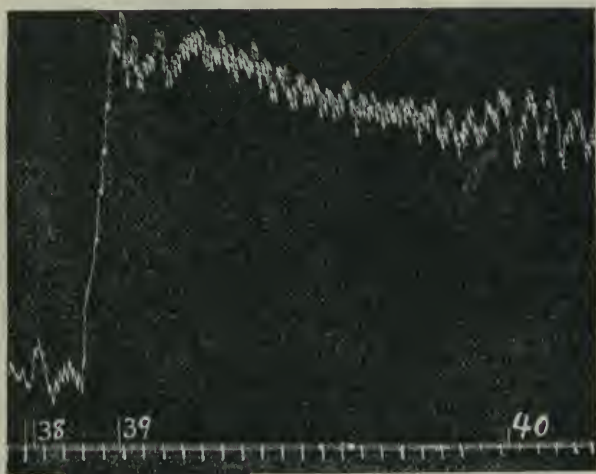


FIG. 21. BLOOD PRESSURE TRACINGS FROM DOG 278

At 38, 0.5 mgm. strychnine was injected intravenously; at 39 the cava pocket was closed and at 40 it was opened; duration of the pocket collection was three minutes. Time trace ten second intervals. Line of zero pressure moved up 35 mm. and the figure then reduced to two-thirds.

and to zero. Increased liberation of epinephrin might have been a minor factor in the preceding rise of pressure. A gradual fall of pressure of 25 mm. of mercury was produced by closing off the cava pocket at the moment when the rise had reached its maximum (fig. 21). We desire to point out clearly that even if no effect were produced upon the pressure by shutting off the adrenal blood, the epinephrin might still have been taking an appreciable share in initiating and sustaining the rise of blood pressure. For the activity of the vasomotor center was obviously

so intense that the withdrawal of the epinephrin from the blood might be easily and immediately compensated for by the nervous mechanism, all the more as it is, of course, impossible by suddenly shutting off the adrenal blood to cause an *instantaneous* disappearance of epinephrin from the circulation. The possibility that vasomotor compensation may mask the epinephrin effect has not been sufficiently considered by writers who have argued that because the clipping of the adrenal veins does not cause an immediate fall of the blood pressure, the normal epinephrin discharge cannot have been exerting any influence whatever upon the pressure. We do not suggest that any of the sympathico-mimetic actions of the naturally secreted epinephrin can ever under ordinary conditions rival in magnitude the actions of the nervous mechanisms; the epinephrin factor must be considered at best a subordinate one. But when on the strength of a supposedly crucial experiment it is denied that epinephrin enters even in the slightest degree into a given physiological reaction, it must be carefully considered whether the experiment is really crucial.

EXPERIMENTS ON CATS

In the cats, as in the dogs, we began with large doses. In the first experiments urethane (1.5 to 1.75 gram per kilogram by stomach tube) was used as the anesthetic. Later on evidence was obtained that urethane is not the most suitable anaesthetic when it is desired to elicit the maximal effect of a given dose of strychnine, owing to its action in diminishing the reflex excitability of the cord. Qualitatively, however, these experiments yielded the same result as those with other anaesthetics.

Condensed protocol; cat 228; female; weight, 2.5 kgm.

Anesthetized with urethane. Obtained a specimen of indifferent blood from the jugular vein. Made cava pocket. Started artificial respiration (good spontaneous respiration). Then collected adrenal blood.

10.45 a.m. First specimen, 2.1 grams in 30 seconds (4.2 grams per minute). Second specimen, 5.2 grams in 90 seconds (3.5 grams per minute).

- 11.00 a.m. Injected 1 mgm. strychnine into jugular vein. Tonic convulsions occurred in about 1 minute.
- 11.02 a.m. Third specimen, 2.15 grams in 30 seconds (4.3 grams per minute). Fourth specimen, 6.7 grams in 120 seconds (3.3 grams per minute).
- 11.05 a.m. Closed abdomen with clamps.
- 11.30 a.m. Reflexes only slightly increased.
- 11.45 a.m. Reflexes not exaggerated. Fifth specimen, 2.4 grams in 30 seconds (4.8 grams per minute). Sixth specimen, 6.75 grams in 120 seconds (3.25 grams per minute).

Obtained another specimen of jugular blood. Combined weight of adrenals 0.492 gram.

The observations on both intestine and uterus segments permitted a good assay. As so many intestine tracings have been already reproduced, only some specimens of the uterus tracings are given in figure 22. With the intestine it was shown that the second specimen (collected before strychnine) was somewhat weaker than 1:5,300,000 adrenalin, and somewhat stronger than 1:6,300,000. The concentration was taken at 1:5,800,000, corresponding to an output of 0.0006 mgm. per minute for the cat, or 0.00024 mgm. per kilogram per minute.

The fourth specimen (collected two and one-half minutes after injection of the strychnine) was found to be stronger than the second. It was assayed at 1:3,300,000. Figure 22 (observations 66 and 68) indicates that it was stronger than 1:3,500,000. The output of epinephrin, taking the fourth specimen at 1:3,300,000 is 0.001 mgm. per minute for the cat, or 0.0004 mgm. per kilogram per minute, i.e., nearly twice as much as the original output.

The sixth specimen (collected forty-five minutes after injection of strychnine) was found both with intestine and uterus to be stronger than the fourth (fig. 22, observations 65 and 66). In observations 50 and 51, the fourth and sixth specimens produced about the same effect upon the uterus segment, but this is because the dilution employed for these two observations was such that even the fourth specimen caused a practically maximal effect. The sixth specimen was assayed at 1:1,650,000

adrenalin, corresponding to an output of 0.002 mgm. per minute for the cat, or 0.0008 mgm. per kilogram per minute, between three and four times the initial output.

In the next experiment a cat of exactly the same weight received the same dose of strychnine as cat 228. It was planned to collect the last specimen at a much longer interval from the strychnine injection, but on account of the deterioration of the circulation it had to be taken in one hour.

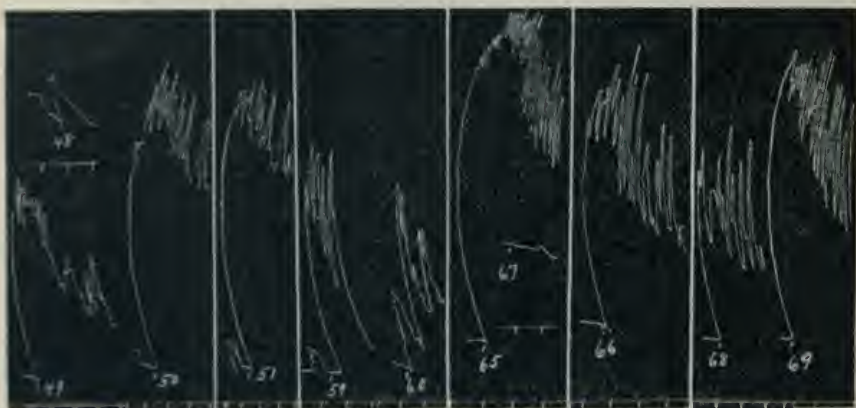


FIG. 22. UTERUS TRACINGS. BLOODS FROM CAT 228

At 48 Ringer was replaced by jugular blood (collected after strychnine injection); at 49 by the second adrenal specimen (collected before strychnine); at 50 by the fourth adrenal specimen (collected immediately after strychnine); at 51 by the sixth adrenal specimen (collected forty-five minutes after strychnine). In observations 48-51 the bloods were diluted with ten volumes Ringer. At 52 Ringer was replaced by the fourth; at 53 by the sixth adrenal specimen (both bloods being diluted with nineteen volumes Ringer); at 54 Ringer was replaced by the sixth; at 55 by the fourth adrenal specimen; at 56 by jugular blood (collected after strychnine); at 57 by the jugular blood made up with adrenalin to a concentration of 1:3,500,000; at 58 by the jugular blood made up with adrenalin to a concentration of 1:1,750,000. In observations 54 to 58 the bloods were diluted with four volumes Ringer, the adrenalin bloods after adding the adrenalin. (Reduced to two-fifths.)

Condensed protocol; cat 239; male; weight, 2.41 kgm.

Anesthetized with urethane. Obtained a specimen of indifferent blood from jugular vein. Made cava pocket. Then collected adrenal blood.

- 11.05 a.m. First specimen, 1.95 grams in 45 seconds (2.6 grams per minute). Second specimen, 4.6 grams in 120 seconds (2.3 grams per minute).
- 11.15 a.m. Blood pressure 87 mm. Hg.
- 11.16 a.m. Started artificial respiration (breathing well, spontaneously).
- 11.19 a.m. Injected 1 mgm. strychnine into jugular vein. Reflexes exaggerated at once, opisthotonos.
- 11.20 a.m. Blood pressure 64 mm. Hg.
- 11.22 a.m. Third specimen, 2 grams in 60 seconds (2 grams per minute). Fourth specimen, 3.5 grams in 120 seconds (1.75 grams per minute).
- 11.27 a.m. Blood pressure 72 mm. Hg. Reflexes increased.
- 11.30 a.m. Closed abdomen with clamps.
- 12.15 p.m. Blood pressure 56 mm. Hg.
- 12.18 p.m. Fifth specimen, 0.75 gram in 40 seconds (1 gram per minute). Sixth specimen, 3.05 grams in 240 seconds (0.76 gram per minute).

Obtained more jugular blood; also arterial (abdominal aorta) blood
Combined weight of adrenals 0.35 gram.

The second specimen (collected before strychnine) produced a much smaller effect on the intestine segment than the fourth (taken four minutes after injection), and the fourth, a much smaller effect than the sixth (taken one hour after the strychnine injection). Typical tracings are reproduced in figure 23. The technical point must be again emphasised, that from the mere inspection of these tracings no conclusion whatever can be drawn as to any increase in the epinephrin output, since the flow was smaller for the fourth than for the second specimen, and much smaller for the sixth than for the fourth. A careful assay was required to determine that at the time of collection of the fourth specimen the output of epinephrin was definitely increased, although only to a small extent, while at the time of collection of the sixth specimen it was more than doubled.

The second specimen was assayed at 1:5,000,000, corresponding to an output per minute of 0.00046 mgm. epinephrin per minute for the cat, or 0.0002 mgm. per kilogram per minute.



FIG. 23. INTESTINE TRACINGS. BLOODS FROM CAT 239

At 8, 10 and 16 Ringer was replaced by indifferent (jugular) blood, and this at 9 by the second adrenal specimen (collected before strychnine); at 11 by the fourth adrenal specimen (collected four minutes after strychnine); at 17 by the sixth adrenal specimen (collected one hour after strychnine). All the bloods were diluted with three volumes Ringer. (Reduced to two-thirds.)

The fourth specimen was assayed at 1:3,000,000, corresponding to an output of 0.0006 mgm. per minute for the cat, or 0.00025 mgm. per kilogram per minute. In this case the reactions of the segment were so definite and so constant in different observations that there could be no doubt that the rate of output was somewhat increased for the fourth specimen. It would be misleading, however, if we did not point out that we cannot in every case, nor indeed in the majority of instances, assay with average segments so closely as to be certain that an output calculated at 0.00025 mgm. per kilogram per minute is really greater than an output calculated at 0.0002 mgm. It is a safe rule in working with such methods of bio-assay to draw no conclusions from apparent small differences in epinephrin output. A difference of 50 per cent on an output of 0.00025 mgm. is usually, a difference of 100 per cent always unmistakable. It is obvious that even with a segment of average sensitiveness and constancy, a good deal must depend upon the absolute concentrations (which themselves depend upon the blood flows) of the various specimens. Where the blood flows of two specimens are the same a comparison of the reactions produced on the segment will sometimes enable the qualitative conclusion to be drawn that one corresponds to a greater epinephrin output than the other, even when the difference may be very small. Still more is this the case when the specimen with the greater concentration has also the greater flow.

The sixth specimen (taken one hour after strychnine when the increase in the reflex excitability had almost disappeared) was shown to be much stronger than 1:2,200,000. As it was so strong it was mixed first with a given proportion of jugular blood and the mixture then diluted with Ringer's solution. In several such observations it was found to be much stronger than 1:1,500,000 and probably somewhat stronger than 1:750,000. It was finally taken at 1:700,000, corresponding to an epinephrin output of 0.0011 mgm. per minute for the cat, or 0.00046 mgm. per kilogram per minute, more than double the initial output. As the concentration was approximately the possible maximum and the blood flow was relatively small, it is probable that the

calculated output is less than would have been obtained at this time with a better flow. With 70 per cent of serum in the blood, the concentration of epinephrin in the serum would be about 1: 500,000, the "possible normal maximum" as estimated on rabbit segments in shed blood.

Strychnine does not, so far as our present experiments show, possess the power of increasing the concentration beyond the normal maximum.

It was not possible, of course, to know whether in this experiment a specimen collected at some time intermediate between the fourth and sixth might not have shown a greater increase in output than the latter.

In the next experiment a somewhat smaller dose of strychnine was employed. It was one of a series of experiments in which the dose was systematically reduced.

Condensed protocol; cat 225; male; weight, 2.05 kgm.

Anesthetized with urethane. Obtained a specimen of indifferent blood from the jugular vein. Made cava pocket. Started artificial respiration. Then collected adrenal blood.

11.30 a.m. First specimen, 1.2 grams in 45 seconds (1.6 grams per minute). Second specimen, 3.7 grams in 180 seconds (1.2 grams per minute).

11.45 a.m. Injected 0.7 mgm. strychnine (in successive doses of 0.3; 0.2; 0.2 mgm). Marked exaggeration of reflexes occurred, but no convulsions.

11.55 a.m. Third specimen, 1.5 grams in 60 seconds (1.5 grams per minute). Fourth specimen, 5.3 grams in 300 seconds (1.06 grams per minute).

12.05 p.m. Closed abdomen with clamps.

12.45 p.m. Fifth specimen, 1.45 grams in 60 seconds (1.45 grams per minute). Sixth specimen, 5.2 grams in 240 seconds (1.3 grams per minute). Reflexes slightly increased.

Obtained another specimen of jugular blood, also a specimen of arterial (abdominal aorta) blood. Combined weight of adrenals 0.292 gram.

Convulsions were not present at any time in this animal, but the reflex excitability was at first markedly increased. The strychnine (0.7 mgm. in all) was injected in successive doses till this occurred. Figure 24 shows that the fourth adrenal blood specimen (collected a few minutes after the last dose of strychnine) caused a much greater inhibition of the intestine segment than the second specimen (collected before strychnine), although the blood flow was only slightly less. The sixth specimen (collected an hour after the first injection of strychnine) had a much greater effect than the fourth, and a very much greater effect

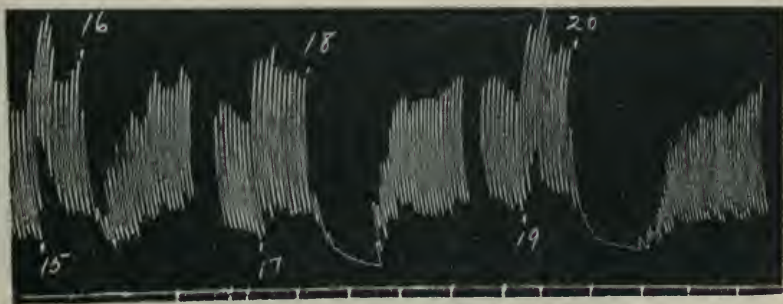


FIG. 24. INTESTINE TRACINGS. BLOODS FROM CAT 225

At 15, 17, and 19 Ringer was replaced by jugular blood, and this at 16 by the second adrenal blood specimen (collected before strychnine); at 18 by the fourth adrenal specimen (collected fifteen minutes after strychnine); at 20 by the sixth adrenal specimen (collected one hour after strychnine). All the bloods were diluted with three volumes Ringer. (Reduced to one-half.)

than the second, although the flow was somewhat greater than for the second specimen. This was confirmed by several other observations. The second specimen was assayed at 1:2,400,000 adrenalin, giving an output of epinephrin of 0.0005 mgm. per minute for the cat, or 0.00025 mgm. per kilogram per minute.

The fourth specimen was assayed at 1:1,600,000, corresponding to an output of 0.0007 mgm. per minute for the cat, or 0.00035 mgm. per kilogram per minute. The assay was such that there was no question that the output was definitely increased at the time of collection of the fourth specimen. The sixth specimen was assayed at 1:1,200,000, corresponding to an

output of 0.001 mgm. per minute for the cat, or 0.0005 mgm. per kilogram per minute, double the original output before strychnine.

In the next experiment, the total dose of strychnine per kilogram of bodyweight was again slightly reduced. The injection was made in successive doses till the reflex excitability became distinctly exaggerated, but there were no convulsions at any stage. Artificial respiration was not required.

Condensed protocol; cat 259; male; weight, 4.1 kgm.

Anesthetized with urethane. Obtained a specimen of indifferent blood from the femoral vein. Made cava pocket. Then collected adrenal blood.

- 11.30 a.m. Blood pressure before collection of adrenal specimen 114 mm. Hg. First specimen, 2.65 grams in 30 seconds (5.3 grams per minute). Second specimen, 9.05 grams in 120 seconds (4.5 grams per minute).
- 11.40 a.m. Injected 1.25 mgm. strychnine into jugular vein, in three doses (0.5, 0.25, 0.5 mgm.) with short intervals between each dose, until reflexes became distinctly exaggerated.
- 11.47 a.m. Blood pressure before collection of third adrenal specimen 128 mm. Hg. Third specimen, 2.1 grams in 30 seconds (4.2 grams per minute). Fourth specimen, 7.85 grams in 150 seconds (3.14 grams per minute).
- 11.55 a.m. Reflexes markedly exaggerated.
- 12.10 p.m. Reflexes still increased, but less marked than at 11.55. Blood pressure 105 mm. Hg.
- 12.15 p.m. Fifth specimen, 1.8 grams in 30 seconds (3.6 grams per minute). Sixth specimen, 8.1 grams in 180 seconds (2.7 grams per minute).
- 12.45 p.m. Blood pressure 60 mm. Hg.
- 12.50 p.m. Seventh specimen, 1.1 grams in 30 seconds (2.2 grams per minute). Eighth specimen, 5.9 grams in 240 seconds (1.5 grams per minute).

Blood obtained from abdominal aorta. Combined weight of adrenals 0.73 gram.

Figure 25 shows that the inhibitory effect produced on the rabbit intestine segment by the second, fourth, sixth, and eighth adrenal specimens increased progressively in the order named. Since, however, there was also a progressive decrease in the blood flow, no conclusion can be arrived at as to any increase in the epinephrin output from a mere comparison of these tracings. The assay proved that the second specimen (collected before strychnine) had a concentration of 1:4,500,000 epinephrin, corresponding to an output of 0.001 mgm. per minute for the cat, or 0.00025 mgm. per kilogram per minute. The fourth

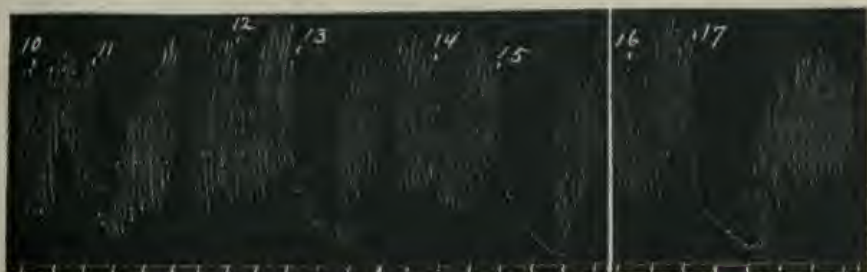


FIG. 25. INTESTINE TRACINGS. BLOODS FROM CAT 259

At 10, 12, 14, and 16 Ringer was replaced by indifferent (femoral vein) blood, and this at 11 by the second adrenal specimen (collected before strychnine); at 13 by the fourth adrenal specimen (collected eight minutes after strychnine); at 15 by the sixth adrenal specimen (collected thirty-five minutes after strychnine); at 17 by the eighth adrenal specimen (collected seventy minutes after strychnine). All the bloods were diluted with three volumes Ringer. (Reduced to one-half.)

specimen (collected as soon as a distinct effect was produced on the reflex excitability, seven minutes after the first dose of strychnine) had a concentration of 1:3,000,000, giving the same output as the second specimen. At this time there was no demonstrable increase in the output. The sixth specimen (collected thirty-five minutes after the first strychnine injection, when the reflex excitability was still exaggerated, although less than before) was assayed at 1:1,250,000, giving an output of 0.002 mgm. per minute for the cat, or 0.0005 mgm. per kilogram per minute, double the initial output before strychnine. The eighth adrenal specimen (collected seventy minutes after the administration of

strychnine, when the respirations had slowed down considerably and the exaggeration of the reflex excitability had largely passed off) was assayed at 1: 700,000 adrenalin, corresponding to an output of 0.002 mgm. per minute for the cat, or 0.0005 mgm. per kilogram per minute, the same as for the sixth specimen and twice the original output before strychnine. The output observed at this time would probably have been greater had the blood flow not diminished so much. For the concentration was at the possible normal maximum. The proportion of serum in the blood was 76.5 per cent, as determined by the electrical method (8) and 73.5 per cent as determined by the haematocrite. The concentration in the serum would, therefore, be 1: 530,000.

In this experiment then the increase in the epinephrin output was as great, and probably greater seventy minutes after the administration of strychnine as thirty-five minutes after.

There is good reason to believe that the smaller effect of strychnine in increasing epinephrin output in cats under urethan than in dogs under morphine and ether is largely at any rate due to the difference in the action of the anesthesia on the nervous system in the two cases. The two following experiments illustrate this point.

In a deeply urethanized cat (238), weighing 1.64 kilogram, the intravenous injection of a large dose of strychnine (1 mgm. of the sulphate) caused no convulsions and no increase in the reflex excitability. The output of epinephrin was trebled five minutes after the injection. A further injection of 2 mgm. of strychnine (in two doses) still caused no convulsions and only a slightly increased reflex excitability. The epinephrin output six minutes after the last injection of strychnine was also about three times the original output before the first dose. An adrenal blood specimen collected about fifty minutes after the last strychnine injection still showed about four times the initial output. The fact that strychnine, which under the conditions of this experiment scarcely affects the motor mechanisms of the cord, causes a substantial and sustained augmentation of the epinephrin output has of course a bearing upon the mechanism of the strychnine action upon epinephrin discharge. As regards the question of

the minimum effective dose of strychnine for increasing the epinephrin liberation, the experiment has at least this significance: it shows that it is not necessary that the excitability of the motor reflex paths should be increased. Other experiments confirm this. A more important deduction, however, for our present purpose can be drawn from a comparison of the increase in epinephrin output produced by the very large amount of strychnine administered intravenously in this experiment, with the increase produced by one-twenty-fourth of the amount per kilogram of bodyweight in cat 308. The maximum increase in cat 308 was five times the initial output, a proportional increase greater than in cat 238. The only difference between the two experiments which can account for this is that cat 308 was anesthetized with ether alone and cat 238 with urethane. It seems clear, then, that the effective doses in the urethane experiments are all far greater than would be effective in an etherized or non-anesthetized animal.

Condensed protocol; cat 238; female; weight, 1.64 kgm.

Anesthetized with urethane. Obtained a specimen of indifferent blood from the jugular vein. Made cava pocket. Started artificial respiration (breathing well, spontaneously. Then collected adrenal blood.

- 10.35 a.m. First specimen, 1.3 grams in 45 seconds (1.7 grams per minute). Second specimen, 5.3 grams in 180 seconds (1.7 grams per minute).
- 10.45 a.m. Injected 1.0 mgm. strychnine into jugular vein. Reflexes were not increased. Deeply anesthetized.
- 10.50 a.m. Third specimen, 1.95 grams in 30 seconds (3.9 grams per minute). Fourth specimen, 4.5 grams in 90 seconds (3.0 grams per minute).
- 11.00 a.m. Injected 1 mgm. strychnine into jugular vein.
- 11.04 a.m. Injected 1 mgm. strychnine into jugular vein.
- 11.07 a.m. Slight increase in reflexes—no convulsions.
- 11.10 a.m. Fifth specimen, 1.25 grams in 30 seconds (2.5 grams per minute). Sixth specimen, 4.35 grams in 120 seconds (2.17 grams per minute).
- 11.15 a.m. Closed abdomen with clamps.

11.50 a.m. Seventh specimen, 1.35 grams in 30 seconds (2.7 grams per minute). Eighth specimen, 3.8 grams in 120 seconds (1.9 grams per minute).

Obtained more jugular blood, and a specimen of arterial (abdominal aorta) blood. Combined weight of adrenals 0.288 gram.

Figure 26 shows that the fourth adrenal specimen is decidedly stronger than the second in spite of the much greater blood flow during collection of the fourth. This, of itself proves that the epinephrin output at the time of collection of the fourth specimen was increased. The sixth specimen caused a somewhat greater inhibition of the intestine segment than the fourth. This was more evident in other observations than in observations 4 and 6, figure 26. The eighth specimen caused the greatest inhibition of all. A detailed assay gave for the second specimen a concentration of 1:5,000,000 epinephrin, corresponding to an output of 0.00034 mgm. per minute for the cat, or 0.0002 mgm. per kilogram per minute. The fourth specimen was taken at 1:2,300,000, denoting an output of 0.0013 mgm. per minute for the cat, or 0.0008 mgm. per kilogram per minute. The eighth specimen was assayed at 1:1,750,000, corresponding to an output of 0.00125 mgm. per minute for the cat, or 0.00075 mgm. per kilogram per minute, practically the same as in the fourth specimen.

Condensed protocol; cat 308; male; weight, 3.33 kgm.

Anesthetized with ether. Obtained a specimen of indifferent blood from the jugular vein. Made cava pocket. Then collected adrenal blood.

10.35	a.m.	Blood pressure 100 mm. Hg.
10.36	a.m.	First specimen, 1.95 grams in 30 seconds (3.9 grams per minute).
10.36½	a.m.	Second specimen, 6.7 grams in 120 seconds (3.35 grams per minute).
10.44	a.m.	Injected 0.25 mgm. strychnine into jugular vein.
10.45	a.m.	Very slight increase in reflex excitability.
10.46	a.m.	Slightly greater increase in reflex excitability.

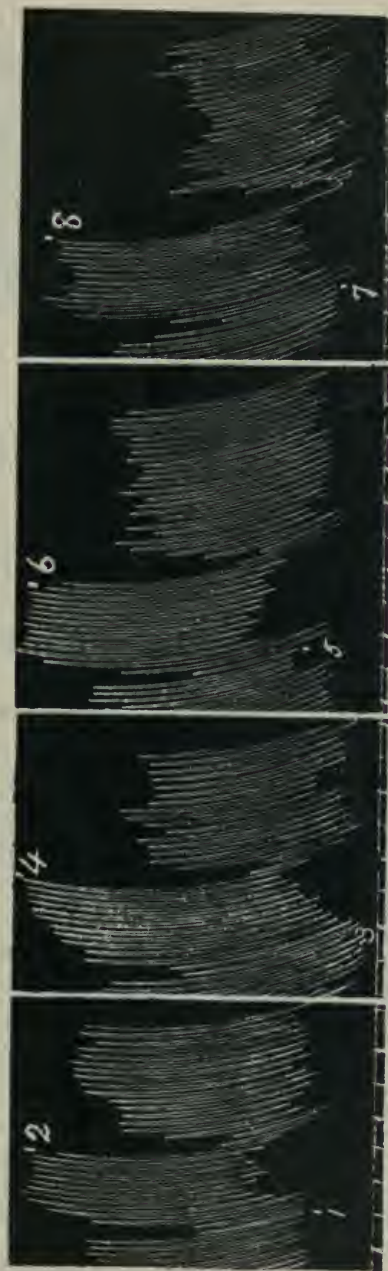


FIG. 26. INTESTINE TRACINGS. BLOODS FROM CAT 238

At 1, 3, 5 and 7 Ringer was replaced by indifferent (jugular) blood, and this at 2 by the second adrenal blood specimen (collected before strychnine); at 4 by the fourth adrenal specimen (collected five minutes after strychnine); at 6 by the sixth adrenal specimen (collected six minutes after two more doses of strychnine); at 8 by the eighth adrenal specimen (collected fifty minutes after the last dose). All the bloods were diluted with three volumes Ringer. (Reduced to one-half.)

- 10.46 a.m. Third specimen, 2.2 grams in 30 seconds (4.4 grams per minute).
- 10.46½ a.m. Fourth specimen, 6.6 grams in 120 seconds (3.3 grams per minute). Blood pressure 86 mm. Hg. Reflexes slightly increased.
- 10.53 a.m. Very little increase in reflex excitability.
- 10.58½ a.m. Fifth specimen, 1.55 grams in 30 seconds (3.1 grams per minute).
- 10.59 a.m. Sixth specimen, 6.9 grams in 150 seconds (2.76 grams per minute). Blood pressure 85 mm. Hg. No increase in reflex excitability.
- 11.31 a.m. Seventh specimen, 1.35 grams in 30 seconds (2.7 grams per minute).
- 11.31½ a.m. Eighth specimen, 6.35 grams in 180 seconds (2.12 grams per minute). Blood pressure 72 mm. Hg. No increase in reflex excitability.

Obtained more venous blood. Combined weight of adrenals 0.473 gram.

The epinephrin assay showed that the second specimen (collected before strychnine) was weaker than the fourth (collected two and one-half minutes after strychnine); the fourth, weaker than the sixth (taken fifteen minutes after the strychnine), and the sixth weaker than the eighth (fig. 27). Since the flows for the second and fourth specimens were the same, the output was already augmented in the fourth specimen and no trace of a preliminary diminution was seen in this experiment. This is instructive, for in another experiment (cat 258) in which the same relatively small dose of strychnine was injected intravenously into a urethanized cat without causing any increase in the reflex excitability, the adrenal blood specimen collected three to four minutes after the strychnine injection had a decidedly smaller concentration of epinephrin (fig. 28, observations 2 and 4, confirmed by other observations) than the specimen obtained before strychnine (1:5,500,000 as compared with 1:4,800,000), although the blood flow was nearly twice as great in the latter. The output of epinephrin was, therefore, distinctly diminished at this time. A given dose of strychnine in a urethanized cat

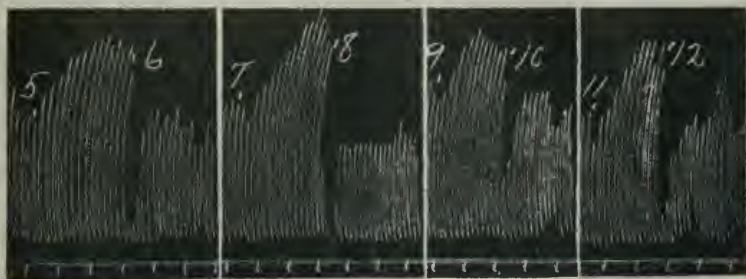


FIG. 27. INTESTINE TRACINGS. BLOODS FROM CAT 308

At 5, 7, 9 and 11 Ringer was replaced by jugular blood, and this at 6 by the sixth adrenal specimen (collected fifteen minutes after injection of strychnine); at 8 by the eighth adrenal specimen (collected fifty minutes after injection of strychnine); at 10 by the second adrenal specimen (collected before injection of strychnine); at 12 by the fourth adrenal specimen (collected two minutes after injection of strychnine). All the bloods were diluted with three volumes Ringer. (Reduced to one-half.)

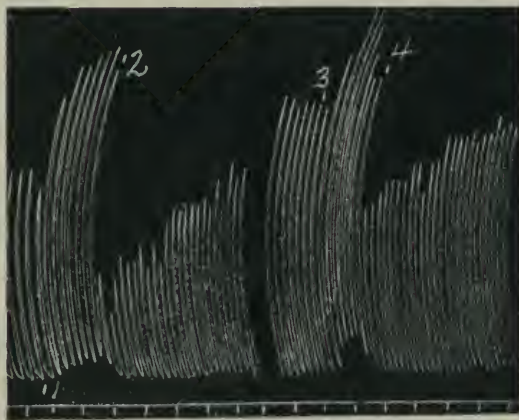


FIG. 28. INTESTINE TRACINGS. BLOODS FROM CAT 258

At 1 and 3 Ringer was replaced by indifferent (femoral vein) blood, and this at 2 by the second adrenal specimen (collected before a small dose of strychnine); at 4 by the fourth adrenal specimen (collected four minutes after the strychnine). The bloods were diluted with three volumes Ringer. (Reduced to one-half.)

would correspond as regards its action on the epinephrin secretion to a smaller dose in an etherized cat.

The second specimen (in cat 308) was found to be decidedly weaker than 1:5,700,000 adrenalin, (confirmed by several observations), and decidedly stronger than 1:8,500,000. It was approximately equal to 1:7,000,000, corresponding to an output of 0.0005 mgm. per minute for the cat, or 0.00015 mgm. per kilogram per minute.

The fourth specimen was much stronger than 1:5,700,000, somewhat stronger than 1:4,300,000. Taking it at 1:4,000,000, the output was calculated at 0.0008 mgm. per minute for the cat, or 0.00025 mgm. per kilogram per minute, nearly twice the initial output. The sixth specimen was assayed at 1:1,200,000, corresponding to an output of 0.0023 mgm. per minute for the cat, or 0.0007 mgm. per kilogram per minute, nearly five times the initial output.

The eighth specimen was assayed at 1:1,000,000, giving 0.0021 mgm. per minute for the cat, or 0.00065 mgm. per kilogram per minute, fully four times the original output. This was nearly fifty minutes after the strychnine injection. The increase in the sixth and eighth specimens was really somewhat greater than stated. For while the second and fourth specimens were assayed in the ordinary routine soon after the bloods were obtained, the assay of the sixth and eighth specimens could not be completed till next day, and although the bloods were kept on ice, some epinephrin must have disappeared. The proportion of serum in the blood was seventy per cent. The serum of the eighth specimen, therefore, contained (twenty-four hours after being drawn) 1:700,000 epinephrin.

GENERAL CONCLUSIONS FROM THE EXPERIMENTS IN WHICH

ADRENAL BLOOD WAS DIRECTLY ASSAYED ON

RABBIT SEGMENTS

In cats, the results were qualitatively the same as in dogs. Quantitatively the maximum increases in the output of epinephrin were in general less in the cat than in the dog. But this was due mainly, if not entirely, to the fact that urethane was the

anesthetic commonly employed for the cats, and with this anesthetic the reflex excitability of the motor mechanisms of the cord was reduced, so that considerably greater doses of strychnine were tolerated without producing convulsions than was the case in the dogs under morphine and ether. Nevertheless, the increases in output in the urethanized cats were quite definite (from two to four times the initial output or even more). In cats, under ether alone, considerably smaller doses of strychnine caused marked effects. As in the case of the dogs, it must be emphasized that doses which caused no convulsions whatever, and only a moderate increase in reflex excitability, were capable of increasing the epinephrin output notably (up to five or six times the original output or more), that the increase persisted a considerable time, that it was often present and sometimes indeed, at its maximum when the heightened motor reflex excitability could no longer be demonstrated.

As regards the length of time during which the increase in the output persists, we have made no attempt to follow it beyond an hour and a half. But at the end of the experiment, it was much more common to find the output still distinctly increased than to find that it had returned to normal, so that it may be concluded with confidence that the effect of a single dose of strychnine is not at all transient.

We have not tried systematically to determine the minimum dose which can produce a definite effect. The necessity of using an anesthetic is, of course, a considerable complication. But since even in anesthetized animals, single doses which reckoned on the bodyweight could be safely used in man have been seen to produce an undoubted and sustained increase in the output, it would seem reasonable to assume that an increased output of epinephrin could be obtained in man by appropriate administration of strychnine, if the production of such an effect should be thought to be indicated for any therapeutic purpose. We express no opinion as to the possible therapeutic applications of the facts described in this paper beyond the suggestion that in such an emergency as cardio-vascular shock, the action of strychnine on the epinephrin output might have some value, of course,

in conjunction with other established methods of treatment, if any methods can be said to be established. It is not without interest that strychnine has been sometimes recommended for treatment of shock without any definite pharmacological basis for the recommendation. Our suggestion is based on indications gradually accumulating in the course of our work, that while the epinephrin output of the adrenals may not under ordinary conditions exert any important action upon the circulation, yet when the circulation is failing, as in shock, and the nervous regulation which plays normally the predominant rôle, has become totally inadequate, the *relative* importance of the epinephrin factor may be enhanced. At this moment a marked and sustained increase in the epinephrin output might intervene, we will not say as an obviously decisive factor, but as a sustaining influence, especially upon the heart, which might help to turn the balance in the right direction.

We have not infrequently had the opportunity to observe that in these circumstances it was especially easy to demonstrate that the normally secreted epinephrin was really not without influence upon the heart and that strychnine seemed to produce an augmentation of this influence.

We do not subscribe to the view expressed by a number of investigators and most recently by Gley (3) that the epinephrin normally liberated from the adrenals cannot be shown to exert any action whatever. It is quite certain that it is not indispensable (19). Nor is there sufficient reason for the view that an augmented epinephrin liberation is a factor in eliciting any of the bodily changes associated with emotional disturbances, or in the increased mobilization of sugar in certain experimental hyperglycemias. But it is going too far, we believe, in the other direction to assert that the naturally secreted epinephrin produces no detectable effect at all. The effects which it does produce are not gross effects which it is easy to detect. Nor is it possible in the present state of our knowledge to assign to any of them a rôle of definite physiological significance. But we have seen, and to some extent studied, two or perhaps three reactions which demonstrate that the amount of epinephrin spontaneously

given off by the adrenals, under our experimental conditions, does exert an appreciable action upon certain structures.

One of these reactions, on the pupil after removal of the superior cervical ganglion, has already been described (7).

The second is the effect upon the heart-beat, which has just been alluded to and which will come more appropriately in another paper. A third reaction, on which further study is necessary, concerns the relation of the normally secreted epinephrin to the rate of the heart. We have obtained some evidence that such changes in the amount and concentration of the naturally secreted epinephrin passing through the coronary vessels as can be produced by altering the distribution of the blood through stimulation of vasomotor nerves and in other ways, can affect the rate of the heart, without any change having been caused in the rate of output of epinephrin (17). Further evidence of a relation of the normal epinephrin output to the heart rate seems to be afforded by a comparison of the effects produced in cats on the rate by section of the accelerantes (excision of the stellate ganglia) after previous section of the vagi, when the adrenal epinephrin is normally entering the circulation and in the absence of epinephrin. It seemed easier to demonstrate a decided slowing of the heart after section of the accelerantes in the absence of an epinephrin secretion. The suggestion is that when the nervous influence is eliminated the stimulation produced by the epinephrin still suffices to maintain the heart rate at a higher level than in the absence of epinephrin. Further work, however, is necessary in regard to this last test.

It is of course possible that if the adrenal medulla or cortex forms and gives off to the blood other and perhaps more important substances than epinephrin, the output of these may also be influenced by the action of strychnine upon the nervous mechanism. Our experiments naturally throw no light upon this, but the possibility should be borne in mind.

Table 1 summarizes the results obtained with segment assays which are not complicated by the repetition of the initial dose of strychnine. In addition to adrenal specimens collected after strychnine in experiments where only one dose was given, speci-

TABLE I

NUMBER OF ANIMAL	WEIGHT kgm.	STRYCHNINE IN- JECTED mgm.	STRYCHNINE PER KILOGRAM	BEFORE INJECTION OF STRYCHNINE						AFTER INJECTION OF STRYCHNINE						Blood col- lected after minutes
				Adrenal blood collected	Length of col- lection seconds	Flow per min- ute grams	Concentration of epineph- rin	Output per minute mgm.	Output per kilogram per minute mgm.	Adrenal blood collected grams	Length of col- lection seconds	Flow per min- ute grams	Concentration of epineph- rin	Output per minute mgm.	Output per kilogram per minute mgm.	
243*	9.5	4.0	0.421	10.0	60	10.0	1: 3,750,000	0.0027	0.00028	12.15	60	12.15	1:2,500,000	0.0048	0.0005	5
246*	7.5	2.0	0.266	7.7	30	15.4	1:13,000,000	0.0012	0.00016	8.3	30	16.6	1:1,500,000	0.011	0.0015	2
243*	4.6	0.5	0.11	9.7	60	9.7	1:13,000,000	0.0008	0.00017	8.9	60	8.9	1:1,250,000	0.007	0.0015	2
263*	4.4	0.25	0.056	6.15	60	6.15	1: 3,800,000	0.0016	0.00036	5.7 5.75 7.4	60 60 120	5.7 5.75 3.7	1:4,300,000 1:2,500,000 1:1,100,000	0.0013 0.002 0.0034	0.0003 0.00045 0.00075	2 20 85
306* †	5.05	2.0	0.4	8.2	60	8.2	1: 8,500,000	0.001	0.0002	16.1 9.55 7.55	60 60 60	16.1 9.55 7.55	1:1,600,000 1:1,200,000 1:4,700,000	0.01 0.008 0.0016	0.002 0.0016 0.0003	10 30 50
309* †	8.1	0.75	0.092	15.9	60	15.9	1: 6,100,000	0.0026	0.00032	11.1 10.3	60 60	11.1 10.3	1:2,300,000 1:2,000,000	0.0048 0.0051	0.0006 0.00063	20 50
225	2.05	0.7	0.35	3.7	180	1.2	1: 2,400,000	0.0005	0.00025	5.3 5.2	300 240	1.06 1.3	1:1,600,000 1:1,200,000	0.0007 0.001	0.00035 0.0005	15 60

228	2.5	1.0	0.4	5.2	90	3.5	1: 5,800,000	0.0006	0.00024	{	6.7	120	3.3	1:3,300,000	0.001	0.0004	2
											6.75	120	3.4	1:1,650,000	0.002	0.0008	45
238	1.64	1.0	0.61	5.3	180	1.7	1: 5,000,000	0.00034	0.0002		4.5	90	3.0	1:3,000,000	0.001	0.0006	5
239	2.41	1.0	0.41	4.6	120	2.3	1: 5,000,000	0.00046	0.0002	{	3.5	120	1.75	1:3,000,000	0.0006	0.00025	3
											3.05	240	0.76	1: 700,000	0.0011	0.00046	60
308	3.33	0.25	0.075	6.7	120	3.35	1: 7,000,000	0.0005	0.00015	{	6.6	120	3.3	1:4,000,000	0.0008	0.00025	2
											6.9	150	2.76	1:1,200,000	0.0023	0.0007	15
											6.35	180	2.12	1:1,000,000	0.0021	0.00065	50

* First 6 are dogs, the others are cats.

† Strychnine administered subcutaneously; all others intravenously.

Cat 308 was anesthetized with ether, the other cats with urethane, the dogs with morphine and ether.

mens collected before repetition of the dose in experiments where it was repeated are included.

Confirmatory evidence that strychnine increases the rate of epinephrin liberation was sought by comparing the effects of given doses of strychnine on the eye, after previous removal of the superior cervical ganglion, in otherwise normal cats and in cats whose epinephrin output had been interfered with by excision of one adrenal and section of the nerves of the other. It has been already pointed out that indirect observations of this kind are more difficult to interpret and, therefore, less trustworthy than assays of the drawn adrenal blood by means of rabbit segments, or than auto-assays of the blood collected in a cava pocket by means of the blood pressure reaction which follows release of the blood into the circulation. We should never think of concluding from the eye observations alone that the output of epinephrin was certainly increased by strychnine, still less of attempting to estimate the amount of the increase. But as confirming the results of the direct methods these observations have a distinct value, for they showed that the (denervated) eye reactions (especially the dilatation of the pupil) induced by strychnine were decidedly greater and lasted much longer in the normal animals than in animals which had been subjected to the adrenal operation.

It must be distinctly pointed out that in the operated cats, the pupil of the denervated eye always remained larger than the pupil of the normal eye, for a longer or shorter time after a strychnine convulsion just as in the normal cats. The difference is not a qualitative, but a quantitative one. It is a pretty general rule that any influence which causes dilatation of the normal pupil (except of course excitation of sympathetic pupillo-dilator fibers) causes a still greater or more permanent dilatation of the pupil on the side from which the superior cervical ganglion has been removed, and the suppression of the epinephrin output does not alter this rule. We have had occasion to observe this in all sorts of experiments. Erroneous conclusions have been drawn by various writers as to the influence of certain factors upon the epinephrin discharge because this has not been taken

into account. Let it be repeated, it is only because of the apparently marked quantitative difference in the eye reactions produced by strychnine in the absence of the epinephrin secretion that we venture to draw any deduction from these observations as to the effect of the drug upon the epinephrin output, and then only by way of corroborating results obtained by more certain methods.

Experiments were made on four cats in which the adrenal operation had been performed ten to twenty-one days previously, and which had completely recovered. The left superior cervical ganglion had been removed at the same time. Three normal cats, from which the left superior cervical ganglion had been removed ten to fifteen days before the strychnine experiments, were used as controls. On two of these normal cats the experiment was repeated on another day, with the same result. All the animals were females. The strychnine was injected subcutaneously. No anesthetic was used, except at the close of the observations on the adrenal cats when the residual epinephrin output was to be determined. The best idea of the difference caused by the adrenal operation can be given by quoting the condensed protocols of a typical experiment from each group.

Condensed protocol; cat 277; female, weight, 1.75 kgm.

Twenty-one days before the experiment the right adrenal was excised, the left adrenal denervated and the left superior cervical ganglion excised. The right adrenal weighed 0.28 gram and contained 0.25 mgm. epinephrin. Left pupil contracted and nictitating membrane forward.

- 10.10 a.m. 0.5 mgm. strychnine injected hypodermically.
- 10.20 a.m. Cat spastic; reflexes markedly exaggerated, excitation brings on spasticity but no convulsions; no change in pupils or nictitating membrane.
- 11.08 a.m. 0.25 mgm. strychnine injected hypodermically.
- 11.12 a.m. Condition same as at 10.20, but more marked; on excitation clonic spasms occurred and during these attacks the left nictitating membrane retracted, the pupils became about equal and maintained equality whether dilatation or contraction was occurring.

- 11.35 a.m. Excited reflexes in cat (tapping) until a tonic convulsion occurred; this at first caused no change from the observations mentioned above, but when asphyxia came on, both pupils dilated to maximum; artificial respiration was at once begun and for 5 to 6 minutes no spontaneous respirations occurred, the heart was very slow and the pupils maximal; as spontaneous respiration came on, the pupils gradually came down, the left remaining slightly wider than the right; the heart rate increased; within 5 to 10 minutes the pupils were equal and finally the left became smaller than the right. On excitation the left pupil became slightly wider than the right, both dilating, but at once came down to its previous condition.
- 12.00 m. Urethane administered; obtained (jugular) indifferent blood; made cava pocket and collected two specimens of adrenal vein blood (blood flow 0.22 gram per minute). The left adrenal weighed 0.293 gram and contained 0.22 mgm. epinephrin. The second adrenal blood specimen assayed at 1:4,000,000, corresponding to an output of 0.00003 mgm. per kilogram per minute, or about one-eighth of the normal average output.

The output of epinephrin determined in adrenal blood collected at the end of the eye observations was greater in this cat than is usual after the adrenal operation. It is not known whether the strychnine was still causing a relatively increased output through fibres which had escaped section at the operation. If so, this would account for the magnitude of the residual liberation. In any case, however, if epinephrin is an important factor in producing and maintaining the great dilatation of the pupil of the denervated eye in the normal animals under the influence of strychnine, the suppression of seven-eighths of the output must be sufficient to cause a great difference in the cat which had undergone the adrenal operation.

Condensed protocol; cat 270; female; weight, 2.5 kgm.

Left superior cervical ganglion excised ten days previously. Left pupil contracted and nictitating membrane forward.

- 2.40 p.m. 0.5 mgm. strychnine injected hypodermically.
2.45 p.m. 0.25 mgm. strychnine injected hypodermically.
2.48 p.m. Tonic convulsion lasting about one-half minute; no artificial respiration was needed. In about 16 seconds from the onset of the convulsion the left pupil became maximal and the nictitating membrane retracted; the right pupil dilated also but not nearly so widely as the left and soon came down again while the left remained maximal for a long while.
4.00 p.m. Cat still spastic; reflexes exaggerated; left pupil much larger than right.
4.30 p.m. Cat quiet, but reflexes are still increased; left pupil is slightly larger than right.
5.00 p.m. Pupils are about equal; when excited, both pupils dilate, but the left becomes somewhat wider than the right, soon coming down to equality again.

EXPERIMENTS ON THE INFLUENCE OF STRYCHNINE ON THE EPINEPHRIN STORE OF THE ADRENALS

We have several times pointed out that changes in the epinephrin store are no certain index of changes in the rate of output of epinephrin. This is very well illustrated by four experiments on cats, in which the effect of the strychnine on the store was investigated. The left adrenal was denervated nine to fourteen days prior to the experiment. In three of the cats the left superior cervical ganglion was also excised, so that the eye reactions could be observed under strychnine. The cats were kept thoroughly under the influence of strychnine for several hours and then killed suddenly by a blow. The adrenals were immediately removed and the amount of epinephrin in each estimated by the colorimetric method of Folin, Cannon and Denis. The doses of strychnine used were much larger than would have been necessary even in anesthetized animals to produce a marked and sustained increase in the epinephrin output and strong convulsions were always induced, artificial respiration by means of the apparatus described by us for use in man (18) being given when necessary to prevent asphyxia during the spasms. As much strychnine was given as was compatible with survival of the

animals for the requisite time. Indeed, a fifth cat died forty minutes after the first dose. Yet in none of the animals could any definite difference, beyond the range of the ordinary variations, be made out in the store of the denervated adrenal as compared with its fellow. In the one experiment made by Elliott (5) the same result was obtained. The formation of epinephrin must, therefore, have been increased in approximately the same measure as its liberation. This is precisely what is seen when the output is increased by stimulation of the splanchnic nerves. It is what might be expected of a secretory nervous mechanism, regulating the output of a substance which is given off constantly to the blood, and we have good evidence that it is through the nervous mechanism and not through any direct effect upon the glands that strychnine increases the epinephrin output. It has been stated in another paper (19) that in an animal in which the epinephrin output has been much diminished or abolished by removal of one adrenal and denervation of the other, strychnine does not cause epinephrin to be liberated although the store in the adrenal is of normal magnitude. That in the absence of the innervation the adrenal medulla is capable of accumulating epinephrin till the normal store is reached, has been clearly demonstrated, first of all by Elliott (5). This, however, is not in any way inconsistent with the speeding up through the nervous mechanism of the process of formation and accumulation when the output is increased.

This speeding up could take place through the intervention of special nerves. Or without this the mere liberation of a small part of the store through the secretory nerves may stimulate the medullary cells to increased accumulation till the normal load has been reached, when in the absence of liberation the accumulation would automatically cease. This would explain why, in the case of the denervated gland, although a depleted store is soon filled up, there is no demonstrable overflow of epinephrin into the blood.

As the experiments on the effect of strychnine upon the epinephrin store in the cats with one superior cervical ganglion excised afforded the opportunity of observing the effect of strychnine

nine upon the denervated eye reactions in animals whose epinephrin output could only be increased half as much as in normal cats, one typical protocol is quoted.

Condensed protocol; cat 261; male; weight, 1.5 kgm.

Left adrenal denervated 12 days before the experiment. Left superior cervical ganglion excised 9 days before the experiment. Left pupil contracted and nictitating membrane forward.

10.20 am. 0.5 mgm. strychnine injected hypodermically.

10.25 a.m. Marked increase in reflex excitability.

10.30 a.m. Tonic convulsion; both pupils dilated widely, but left more than right; after the convulsion the right pupil came down, but the left remained maximal.

10.35 a.m. Left pupil wider than right and nictitating membrane forward; right nictitating retracted.

10.40 a.m. Pupils about equal; cat still spastic; excitement does not cause the left pupil to become larger than the right.

10.45 a.m. Left pupil smaller than right; remains smaller than right when both dilate on excitation of animal.

11.50 a.m. 0.25 mgm. strychnine injected hypodermically.

11.55 a.m. Tonic convulsion; same phenomena as described under 10.30 observation occurred.

1.40 p.m. Effect of strychnine apparently worn off; 0.25 mgm. strychnine injected hypodermically.

1.45 p.m. Tonic convulsion with same phenomena as described above.

3.30 p.m. 0.25 mgm. strychnine injected hypodermically.

3.35 p.m. Tonic, then clonic spasm; same phenomena observed.

4.00 p.m. Still quite spastic; killed by a sudden blow on head.

Left adrenal weighed 0.18 gram and contained 0.19 mgm. epinephrin. Right adrenal weighed 0.16 gram and contained 0.15 mgm. epinephrin.

In these cats the effects on the denervated eye seemed to be greater and more lasting than in the cats with one adrenal removed and the other denervated, but smaller and more transient than in the normal cats.

The results of the experiments on the effect of strychnine on the epinephrin store are given in table 2.

TABLE 2

NUMBER OF ANIMAL	WEIGHT	WEIGHT OF ADRENALS		EPINEPHRIN		TIME AFTER OPERA- TION	STRYCHNINE	
		Left	Right	Left	Right		Total dose	Duration of action.
	<i>kgm.</i>	<i>grams</i>	<i>grams</i>	<i>mgm.</i>	<i>mgm.</i>	<i>days</i>	<i>mgm.</i>	<i>hours</i>
251	2.16	0.14	0.15	0.16	0.16	12	1	5
253	2.87	0.26	0.248	0.22	0.18	14	1.25	5
261	1.5	0.18	0.16	0.19	0.15	12	1.25	6
271	2.1	0.147	0.15	0.17	0.17	9	0.75	$\frac{1}{2}$
272	1.32	0.108	0.12	0.12	0.12	9	0.5	4

SUMMARY

1. The paper begins with a discussion of essential points in the technique of measuring the epinephrin output. It is pointed out that in general it is no more possible to demonstrate (or measure) alterations in the rate of epinephrin output by observations which only take account of changes in the concentration of epinephrin in the blood coming from the adrenals, while ignoring concomitant changes in the rate of the blood flow, than it would be to demonstrate (or measure) alterations in the rate of carbon dioxide production in an organ by observations which only took account of the number of volumes of carbon dioxide in 100 cc. of blood, but paid no attention to the number of cubic centimeters of blood passing through the organ in a given time. In this connection it is again shown that statements in the literature as to the influence of various conditions in notably augmenting the rate of epinephrin output are vitiated by neglect of this factor.

2. Strychnine causes a marked increase in the output of epinephrin from the adrenals (in the dog and cat). Although it is only by chance that a sample of adrenal blood corresponding to the maximum increase can be collected, outputs ten times the original output have been observed.

3. The increase is not transient but persists for a considerable time. No attempt was made to continue the experiments until it had completely subsided, as it was not thought that any useful purpose could be served by keeping the animals, after the necessary operation, several hours under the anesthetic. The last

adrenal sample was usually taken an hour to an hour and a half after the strychnine injection and it was the rule to find that at this time the epinephrin output was still notably augmented. Indeed, with the smaller doses the effect may go on increasing during the whole experiment and the last specimen may correspond to a rate of output as great as or greater than that of any previous specimen. Abundant evidence has been produced in other papers that animals under similar experimental conditions, but without strychnine, do not show an increased epinephrin output.

4. No attempt was made to fix a minimum effective dose but it was clearly shown that doses of strychnine well within the therapeutic range, and which caused little or no exaggeration of reflex excitability are capable of producing a considerable augmentation in the rate of output. In this connection it must be remembered that the animals were necessarily well anesthetized, and it is to be supposed that still smaller doses would suffice in non-anaesthetised animals.

5. Indications were obtained in some experiments that the stage of prolonged augmentation of the rate of output, which constitutes the principal action of the drug, may be preceded by a transient diminution. This phenomenon was best seen with the smaller doses and with subcutaneous administration of the drug, presumably because with the larger doses and with intravenous injection the augmentation of the output comes on so rapidly as to mask any preliminary decrease.

6. The augmentation of the output caused by strychnine is associated with a more or less marked increase in the epinephrin concentration, even when at the same time the rate of blood flow through the adrenals has been increased, a phenomenon not seen in the absence of the drug. But no evidence has been found that under the influence of strychnine the possible normal maximum concentration in the plasma (something like 1:500,000 as assayed by rabbit segments in adrenal blood from non-strychninised animals) can be increased.

7. The above conclusions are all based on assays of adrenal blood with rabbit intestine and uterus segments. But corroboration

rative evidence of the augmenting influence of strychnine was obtained by studying the effects produced on the blood pressure by adrenal blood, collected in a cava pocket for a given time before and after strychnine, when the blood was allowed to pass from the pocket into the circulation, and in other ways.

8. In spite of the greatly increased output of epinephrin caused by strychnine, there was no evidence that the epinephrin store of the adrenals is distinctly diminished even by the prolonged action of the drug in large and repeated doses. The accumulation of epinephrin in the glands is therefore increased as well as its liberation. This is what happens during stimulation of the splanchnic except when intermittent stimulation is continued for very long periods. It corroborates other evidence that the strychnine effect is produced by an intensification of the secretory process through the nervous mechanism which normally governs it. There is no direct action on the glands.

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THE ACTION OF DRUGS ON THE OUTPUT OF EPINEPHRIN FROM THE ADRENALS

II. CONCENTRATED SALT SOLUTIONS (SODIUM CAR- BONATE) INJECTED INTO THE CIRCULATION

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Our attention was accidentally drawn to the action upon the epinephrin output of small quantities of concentrated salt solutions introduced directly into the circulation of cats. We were using sodium carbonate solution (half to three-quarters saturated) in the tube connecting the carotid with the mercurial manometer. We were determining the epinephrin output under conditions in which a considerable fall of blood pressure was liable to occur some time after the collection of the adrenal blood specimens on which the normal or initial epinephrin output was estimated. In occasional experiments we were puzzled to find that one or more of the subsequent adrenal specimens had epinephrin concentrations so much out of line with the usual rule, that the concentration is inversely proportional to the blood flow, that it was necessary to assume that the rate of epinephrin liberation had undergone an abrupt and decided change. This had not occurred in long series of experiments made under other conditions in which blood pressure tracings were not necessary. On looking into the matter it was observed that when anomalous behavior of the epinephrin output occurred the fall of blood pressure was speedily followed by an abrupt rise associated with evidence of excitation of the central motor mechanisms (increased reflex excitability and convulsions and changes in the

respiration). It is known that the intravenous injection of concentrated salt solutions leads to those symptoms (1), (2), (3). It seemed fairly clear, then, that small quantities of the carbonate solution passing back from the manometer connection into the artery must have been responsible not only for the motor and vasomotor excitation, but also for the changes in the epinephrin output, presumably through an action on the central nervous mechanism which governs it.

For instance, in one experiment (cat 208) in which it had been intended to follow the epinephrin output in vascular shock, on taking the clip off the carotid in order to begin a blood pressure tracing before the first adrenal specimen was collected the blood pressure was lower than expected and a little of the carbonate solution entered the artery. The blood pressure rose rapidly from 100 to 188 mm. of mercury, with symptoms of excitation of the cord and bulb. The adrenal specimen now collected (the second) was shown, by an assay on rabbit segments, to have a concentration of epinephrin greater than 1:3,300,000, less than 1:1,700,000, and approximately equal to 1:2,500,000, corresponding to an output of 0.0016 mgm. per minute for the cat, or 0.00065 mgm. per kilogram per minute. This is two to three times the normal average, and although no specimen had been obtained before the entrance of carbonate with which to compare it, there is no doubt that the output at the time of collection of the second specimen had been decidedly increased. The fourth adrenal specimen (collected about an hour thereafter, when the blood pressure was only 40 mm. of mercury), had a concentration of 1:2,500,000, the same as that of the second specimen, although the blood flow was not more than one-eighth of that during collection of the second specimen, and the output per kilogram per minute was only 0.00008 mgm.

As a specimen of adrenal blood was not obtained before the entrance of the carbonate, it is impossible to know whether at the time of collection of the fourth specimen the output had regained the initial amount or was still below it.

Condensed protocol. Cat 208; male; weight, 2.4 kgm.

Anesthetized with urethane. Obtained specimen of indifferent blood from jugular. Made cava pocket.

10.22 a.m. Blood pressure 100 mm. Hg. On opening the carotid clip to take the blood pressure some saturated carbonate solution entered the artery and at once the blood pressure rose to 188 mm. Artificial respiration was started and kept up throughout the experiment.

10.26 a.m. Blood pressure 160 mm. Hg. Collected adrenal blood. First specimen, 3.6 grams in 45 seconds (4.8 grams per minute). Second specimen, 10.1 grams in 150 seconds (4.04 grams per minute).

11.13 a.m. Blood pressure 50 mm. Hg. Collected adrenal blood from pocket. Third specimen, 0.9 gram in 60 seconds (0.9 gram per minute). Fourth specimen, 4.1 grams in 480 seconds (0.5 gram per minute).

Obtained more indifferent (venous) blood. Combined weight of adrenals 0.467 gram.

On putting the matter to an experimental test it proved easy to demonstrate that the injection of small quantities of a strong solution of the salt into the blood stream caused a distinct increase in the epinephrin output, simultaneous with the rise of blood pressure and the convulsions. The cardio-inhibitory center was excited and sometimes the vomiting center. The apnoea which is known to follow the intravenous administration of the carbonate was guarded against by artificial respiration, so that asphyxia played no part in the phenomena. As was to be expected, owing to the greater concentration in which the salt arrives at the nervous centres, the greatest effects were elicited by intraarterial injection. A typical experiment (cat 211) follows.

Condensed protocol. Cat 211; female; weight, 2.8 kgm.

Anesthetized with urethane; cannula in carotid for blood pressure.

11.10 a.m. Cava pocket made with cannula pushed into it through renal vein (left) for collection of adrenal blood. The abdominal aorta was not tied off.

- 11.35 a.m. Collected first adrenal specimen, 1.75 grams in 60 seconds (1.75 grams per minute). Second adrenal specimen, 3.5 grams in 240 seconds (0.9 gram per minute).
- 11.45 a.m. Obtained indifferent (jugular) blood.
- 12.04 p.m. Started artificial respiration and kept it up throughout experiment. Inserted cannula into lower end of pocket; clipped off abdominal aorta. Blood pressure before injection of carbonate 90 mm. Hg. Injected *via* carotid artery 5 to 6 cc. half-saturated carbonate solution. Blood pressure just after injection of carbonate 145 mm. Hg. The blood pressure rose to 228 mm. and a third specimen of adrenal blood was at once collected while blood pressure was 200 mm., and a fourth specimen while blood pressure was 180 mm. Hg. Third adrenal specimen, 6.4 grams in 90 seconds (4.2 grams per minute). Fourth adrenal specimen, 7.55 grams in 210 seconds (2.3 grams per minute).

Indifferent blood was now obtained from the abdominal aorta. On dilution with Ringer the arterial blood clotted readily, but only with difficulty before dilution. Combined weight of adrenals 0.580 gram.

The assay showed that the second adrenal specimen (collected before injection of carbonate) was stronger than 1:3,600,000, somewhat weaker than 1:1,800,000. It was finally taken at 1:2,000,000 epinephrin, equivalent to an output of 0.00045 mgm. per minute for the cat, or 0.00016 mgm. per kilogram of body-weight per minute. The fourth adrenal specimen (collected about two minutes after injection of the carbonate) was found to be stronger than 1:1,400,000, and weaker than 1:900,000 adrenalin. It was assayed at 1:1,200,000 corresponding to an output of 0.002 mgm. per minute for the cat, or 0.0007 mgm. per kilogram per minute. At this time the output was accordingly increased three to four times. The third adrenal specimen, collected just before the fourth, was somewhat stronger than the latter, although the blood flow during its collection was almost twice as great. The output of epinephrin at this time must accordingly have been at least eight times the original output before sodium carbonate was administered. As in the case of the other substances investigated (strychnine, nicotine, etc.),

indifferent blood, obtained after injection of carbonate, was always used in the assay of the adrenal blood specimens taken after carbonate administration, as well as the indifferent (venous) blood drawn at the beginning of the experiments. The carbonate indifferent blood was always obtained from a vein, except when the pressure had sunk too low at the end of an experiment, when it was taken from an artery.

In the next experiment (cat 214) the carbonate was injected not directly into an artery, but into the jugular vein. At the same time a smaller dose was employed, namely 1 cc. of the saturated solution.

Condensed protocol. Cat 214; male, weight, 2.35 kgm.

Anesthetized with urethane. Obtained indifferent blood from jugular. Made cava pocket.

10.46 a.m. Artificial respiration started and kept up throughout experiment.

10.48 a.m. Collected adrenal blood. First specimen, 2.4 grams in 60 seconds (2.4 grams per minute). Second specimen, 7.0 grams in 180 seconds (2.3 grams per minute). Blood pressure 84 mm. Hg.

10.58 a.m. Injected 1.0 cc. saturated solution sodium carbonate into external jugular vein. Blood pressure 85 mm. Hg. Gasping and vomiting movements.

11.00 a.m. Third adrenal specimen, 3.45 grams in 45 seconds (5.0 grams per minute). Fourth adrenal specimen, 6.65 grams in 120 seconds (3.3 grams per minute). Blood pressure during collection of third specimen 158 mm. Hg. Blood pressure during collection of fourth specimen 136 mm. Hg.

11.10 a.m. Obtained more jugular blood.

11.57 a.m. Fifth adrenal specimen, 1.2 grams in 60 seconds (1.2 grams per minute). Sixth adrenal specimen, 4.2 grams in 240 seconds (1.05 grams per minute). Blood pressure 56 mm. Hg.

Indifferent blood obtained from abdominal aorta. Combined weight of adrenals 0.348 gram.

Naturally the rise of blood pressure did not begin so soon as with intraarterial injection (fig. 1). nor was it so abrupt. After a moderate initial rise (to a maximum of 124 mm. of mercury) a marked inhibition of the heart ensued, accompanied by a brief, but great fall of pressure (to 26 mm.) This was at once succeeded by a great increase of pressure (to a maximum of 188 mm. of mercury). Asphyxia was completely excluded by artificial respiration, begun before injection of the carbonate. The blood pressure maintained itself at a high level for about two minutes. The three or four considerable and sudden depressions of the curve at this time were concomitant with gasping and vomiting movements. The beginning of collection of the third adrenal blood specimen was at 3, and forty-five seconds later the collection of the fourth specimen was begun (three minutes after injection of the carbonate), the blood pressure being still 135 mm. of mercury.

The assay showed that the second adrenal specimen (collected before injection of the carbonate) had a concentration greater than 1:3,500,000, less than 1:1,700,000. It was finally assayed at 1:2,500,000 epinephrin, equivalent to an output of 0.0009 mgm. per minute for the cat, or 0.0004 mgm. per kilogram per minute. The fourth adrenal specimen (collected three minutes after administration of the carbonate, or two and a half minutes if allowance is made for the dead space in the cannula and cava) was found to be stronger than the second specimen, in spite of the greater blood flow. It was weaker than 1:1,300,000 adrenalin, much stronger than 1:3,500,000, approximately equal to 1:1,700,000, corresponding to an output of 0.002 mgm. per minute for the cat, or 0.00085 mgm. per kilogram per minute, about twice the original output before the carbonate was injected. The sixth specimen (collected an hour after injection of carbonate when the blood pressure was only 56 mm. of mercury) was shown to be somewhat stronger than 1:850,000, and weaker than 1:700,000 adrenalin. It was taken at 1:800,000, corresponding to an output of 0.0012 mgm. per minute for the cat, or 0.0005 mgm. per kilogram per minute, about the same as for the second specimen. With 71 per cent of serum in the



FIG. 1. BLOOD PRESSURE TRACINGS

Cat 214. 2, beginning of carbonate injection; 3, beginning of collection of third adrenal specimen; 4, beginning of collection of fourth adrenal specimen. Time trace, ten second intervals. Line of zero pressure coincides with time trace. (Reduced to two-thirds.)

blood, the concentration of the serum in the sixth specimen would be 1: 500,000, the "possible normal maximum concentration." It has not been shown whether this maximum can be transcended during the increased liberation of epinephrin caused by carbonate injection.

Figure 2 shows that the fourth specimen was stronger than the second, despite the considerably greater blood flow when the fourth was collected. This of itself would prove that the output of epinephrin was increased at this time. The sixth specimen had a much greater concentration than the fourth (fig. 2, observation 6), but as the flow was only one-third as great, nothing can be deduced from the concentration alone as to the output. As already stated, it was shown by the assay that it was not much more than half as great when the sixth specimen was being taken as when the fourth was being collected.

Although there could be little doubt that the effect of the carbonate on the epinephrin output was due to the stimulating action of a strong salt solution on the nervous mechanism which governs the secretion, the possibility could not be ignored that the production of a marked alkalosis might itself more directly affect the rate at which epinephrin was liberated from the adrenals.

In the next experiment (cat 212) a quantity of carbonate a little greater, reckoned on the bodyweight, than that given to cat 214, was injected into the jugular vein, but instead of 1 cc. of the saturated solution, 10 cc. of a 5 per cent solution was administered.

Condensed protocol. Cat 212; female; weight, 2.2 kgm.

Anesthetized with urethane. Obtained indifferent blood from jugular. Made cava pocket.

12.05 p.m. Collected adrenal blood. First specimen, 2.3 grams in 60 seconds (2.3 grams per minute). Second specimen, 4.3 grams in 180 seconds (1.43 grams per minute). Started artificial respiration and kept it up throughout experiment.

12.18 p.m. Injected 10 cc. of 5 per cent sodium carbonate solution into jugular vein; gasping respirations.

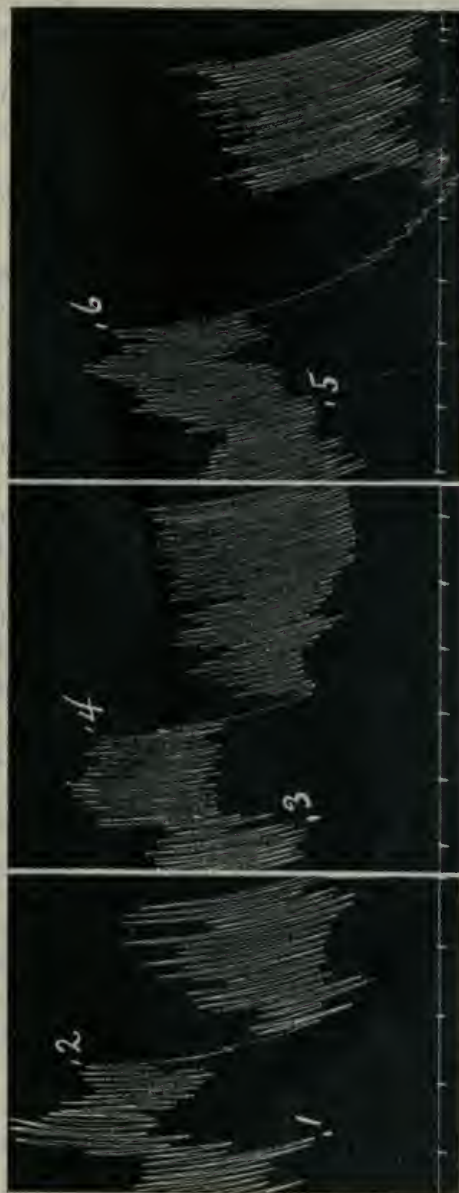


FIG. 2. INTESTINE TRACINGS

Bloods from cat 214. At 1, 3, and 5 Ringer was replaced by jugular blood and this at 2 by the second adrenal specimen (collected before carbonate injection); at 4 by the fourth adrenal specimen (collected three minutes after carbonate injection); at 6 by the sixth adrenal specimen (collected one hour after carbonate injection). All the bloods were diluted with 3 volumes Ringer. Time trace half minutes. (Reduced to two-thirds.)

12.20 p.m. Third adrenal specimen, 2.55 grams in 60 seconds (2.55 grams per minute). Fourth adrenal specimen, 8.45 grams in 240 seconds (2.11 grams per minute).

12.40 p.m. Fifth adrenal specimen, 2.3 grams in 60 seconds (2.3 grams per minute). Sixth adrenal specimen, 8.85 grams in 240 seconds (2.21 grams per minute).

Indifferent blood obtained from the abdominal aorta. Combined weight of adrenals 0.496 gram.

The result was quite different. Although an effect was produced upon the respiration, no definite increase in the output of epinephrin was made out in any of the adrenal blood specimens.

The epinephrin assay showed that the second adrenal specimen (taken before carbonate injection) was stronger than 1:4,000,000, somewhat weaker than 1:3,000,000. It was taken at 1:3,200,000, corresponding to an output of 0.00045 mgm. per minute for the cat, or 0.0002 mgm. per kilogram per minute.

The fourth specimen (collected three minutes after the beginning of the carbonate injection) had a concentration not very different from that of the second. It was assayed at 1:3,500,000, equivalent to an output of 0.0006 mgm. per minute for the cat, or 0.00027 mgm. per kilogram per minute. The sixth specimen (collected twenty-three minutes after injection of carbonate) had the same concentration as the fourth, corresponding also to an output of 0.00027 mgm. per kilogram per minute. The fourth specimen showed a slightly increased output, but the change, if present, was not clearly beyond the limits of error of such determinations.

In the next experiment (cat 213) three times as much carbonate as was used in cat 214 was injected, but in the form of 30 cc. of the 5 per cent solution, in three injections of 10 cc. each into the jugular vein, administered over a period of ten minutes.

Condensed protocol. Cat 213, male; weight, 2.675 kgm.

Anesthetized with urethane. Obtained indifferent blood from jugular. Made cava pocket.

11.10 a.m. Collected adrenal blood. First specimen, 3.75 grams in 60 seconds (3.75 grams per minute). Second specimen, 9.8 grams in 180 seconds (3.3 grams per minute).

- 11.15 a.m. Started artificial respiration and kept it up throughout rest of experiment.
- 11.20 a.m. to 11.30 a.m. 30 cc. of 5 per cent sodium carbonate injected into external jugular vein; in 10 cc. injections; with last injection the pupils became wide and cat gasping, but pupils soon came back to previous condition.
- 11.33 a.m. Third adrenal specimen, 2.55 grams in 60 seconds (2.55 grams per minute). Fourth adrenal specimen, 7.9 grams in 180 seconds (2.63 grams per minute).
- 12.07 p.m. Fifth adrenal specimen, 1.75 grams in 60 seconds (1.75 grams per minute). Sixth adrenal specimen, 4.6 grams in 180 seconds (1.53 grams per minute).

Obtained additional indifferent blood from jugular. Combined weight of adrenals 0.468 gram.

The second adrenal specimen (collected before injection of carbonate) had a concentration of 1:3,500,000, equivalent to an output of epinephrin of 0.00095 mgm. per minute for the cat, or 0.00035 mgm. per kilogram per minute. The fourth specimen (collected four minutes after the end of the carbonate injection) was slightly stronger than the second, but the flow was correspondingly smaller. Taking the fourth specimen at 1:3,000,000, we get 0.0009 mgm. for the cat, or 0.00033 mgm. per kilogram per minute. The sixth adrenal specimen (collected thirty-eight minutes after the end of the carbonate injection) assayed at 1:1,000,000 epinephrin, corresponding to an output of 0.0015 mgm. per minute for the cat, or 0.00055 mgm. per kilogram per minute.

In this experiment, then, no increase in the epinephrin output could be demonstrated in the specimen taken soon after the administration of carbonate. A remote specimen (the sixth) collected thirty-eight minutes after the carbonate injection gave an increase in the output of about 50 per cent.

We do not, of course, conclude that the reaction of the blood or the amount of the alkali reserve exerts no influence upon the rate of liberation (or of formation) of epinephrin. The available methods could not possibly detect small changes. For this reason it was not considered worth while to determine the alteration

in the H-ion concentration of the blood produced by the administration of carbonate. Neither did it enter into our plan to extend the investigation to concentrated solutions of other sodium salts or of dextrose or similar substances. Our object was sufficiently attained when the seemingly anomalous results which were the starting point of the investigation had been traced to the entrance of small quantities of the concentrated sodium carbonate into the circulation, and when the action upon the epinephrin output had been correlated by its time relations and in other ways with a general stimulating action of the solutions upon the bulbo-spinal centres. As the mechanism of the similar, but more intense, much more prolonged and apparently more interesting and important reactions produced by strychnine was being studied in detail (4), (5), it was not judged worth while, at least at present, to define more exactly the seat and mode of action of the concentrated salt solution. Some indications were obtained that in addition to increasing the output of epinephrin, injection of concentrated sodium carbonate solution may at a certain stage produce the opposite effect. But with the relatively small number of experiments performed it was not possible, as in the case of strychnine, to be sure that this action necessarily preceded the stimulating action, nor were the indications of an inhibitory or depressant action so clear. It is, of course, possible that the remote action of the carbonate in virtue of the alkalosis or through some other toxic effect upon the organism, may cause a diminution in the epinephrin output unconnected with the effects of the primary stimulation of the nervous mechanism.

One experiment was made to test whether any marked effect was produced by intravenous injection of concentrated sodium carbonate solution upon the epinephrin store of the adrenals.

Condensed protocol. Cat 218; female; weight, 3.4 kgm.

10.00 a.m. Anesthetized with urethane.

11.20 a.m. Excised right adrenal.

11.25 a.m. Blood pressure 146 mm. Hg.

- 11.26 a.m. Injected 2 cc. sodium carbonate solution (half to three-quarters saturated), intravenously; respiration stopped; started artificial respiration, which was kept up throughout the rest of the experiment. The blood pressure fell gradually during 4 minutes to 72 mm.; then suddenly began to ascend rapidly, reaching a maximum of 235 mm. It remained at about 218 mm. for 3 minutes then again suddenly mounted to about 280 mm. where it remained for 3 to 4 minutes, then gradually fell to 120 mm.
- 12.00 m. Excised left adrenal; blood pressure 98 mm.
- 12.05 p.m. Injected 2 cc. carbonate solution—intravenously. Blood pressure at once fell from 94 to 85 mm., then suddenly rose to a maximum of 160 mm.; then gradually fell to 90 mm. in 4 minutes.
- 12.15 p.m. Blood pressure again mounted, without apparent cause, reaching a maximum of 140 mm.; then gradually fell to 60 mm. in about 5 minutes. Within the next few minutes the pressure fell to zero, and the cat died.

Right adrenal weighed 0.211 gram and contained 0.28 mgm. epinephrin. Left adrenal weighed 0.206 gram and contained 0.21 mgm. epinephrin.

As it was not practicable to use a non-anesthetized animal, one adrenal was excised from a urethanized cat and the carbonate injected into the jugular vein. An enormous rise of blood pressure (to 280 mm. of mercury), ensued after a preliminary fall very much as in cat 214, the chief difference being that the initial minor rise of pressure was absent and that the period which elapsed between the injection and the commencement of the main rise was much longer. The increase in the blood pressure was also more sustained. The increased output of epinephrin during this period did not make any serious inroad upon the epinephrin store of the remaining adrenal. For when it was excised forty minutes later than the first, the store was not diminished more than is usually seen in such an experiment under urethane anesthesia without carbonate injection. The interval between the removal of the two adrenals was purposely made relatively short, so that the diminution associated with the

experimental conditions might not mask completely any more rapidly developed effect due to the carbonate.

While it is not possible from this experiment to determine whether an increased liberation of epinephrin from the one adrenal remaining took any sensible share in the great rise of blood pressure, it was shown clearly by injecting carbonate after removal of the second adrenal that a good rise of pressure was obtained in the absence of epinephrin liberation. Naturally the absolute amount of the rise was less than after the previous injection, as the condition of the animal, of course, had deteriorated. Our experiments on strychnine indicate that even the greater and much more sustained increase in epinephrin output produced by that drug can play but a minor rôle in the increase of arterial pressure.

A point of technique of some importance follows from the above observations. Concentrated solutions of salts must not be employed in the connections of the artery with a mercurial manometer if blood pressure tracings are being taken in experiments on the epinephrin output. If such substances are used, and the conditions of the experiment involve considerable changes of blood pressure, as in experimental shock, e.g., the greatest care must be taken that none of the solution passes into the artery. We abandoned carbonate as soon as our suspicions were confirmed and reverted to sodium citrate solutions (2 per cent). Pains are taken even with the citrate, to prevent any of the solution from entering the circulation, by adjusting the pressure in the manometer from time to time so that it is always a little below the arterial pressure.

SUMMARY

1. Intravascular injection of small volumes of concentrated salt solutions (sodium carbonate) causes a temporary increase in the rate of liberation of epinephrin from the adrenals.

2. This increase is presumably due to stimulation of the nervous mechanism which governs the epinephrin output since it is accompanied by symptoms of a general excitation of the

bulbo-spinal centers, and is not obtained, or only in a minor degree, when even larger quantities of the carbonate are injected in more dilute form.

3. In experiments on epinephrin output, it is not advisable to use concentrated solutions of salts in tubes connecting an artery with a mercurial manometer.

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THE ACTION OF DRUGS ON THE OUTPUT OF EPINEPHRIN FROM THE ADRENALS

III. NICOTINE

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INTRODUCTION

Of the few statements in the literature as to the action of nicotine upon the output of epinephrin, none so far as we are aware, contains any quantitative data whatever. Nor is anyone of them based upon a method capable of yielding direct and unequivocal qualitative evidence. The best investigation is that of Dale and Laidlaw (1). But they merely observed that certain reactions which are elicited by nicotine on the non-pregnant uterus of the cat and in the eye after removal of the superior cervical ganglion are modified when the experiment is made under such conditions that epinephrin can no longer reach these structures from the adrenals. They explain the difference by the hypothesis that the nicotine action is in part due to a stimulation of the adrenals to increased liberation of epinephrin. We believe that indirect evidence of this kind, as has been set forth in another paper on the action of strychnine upon the epinephrin output (2), is valuable when it corroborates the results of more direct methods, but that when standing alone it must be interpreted with great care. For example, it would not be possible in these experiments to know whether as a consequence of the action of the drug on the circulation a larger amount of epinephrin per minute or a greater concentration of it might not be supplied to the uterus or the eye without any change whatever having occurred in the rate of discharge. It is scarcely neces-

sary to add that in any case such observations, if it be granted that they indicate a stimulating effect of nicotine upon the epinephrin output, do not afford the means of measuring the amount of the increase.

These remarks are not intended as a criticism of these interesting and suggestive experiments, they are meant merely to point out the limitations of such indirect methods.

Gley's experiment (3) on the effect of nicotine upon the blood pressure before and after excision of the adrenals in animals with the spinal cord destroyed, besides being also a purely qualitative experiment, loses most of its weight by proving too much. He gives a figure showing a rise of pressure from less than 30 mm. to 140 mm. of mercury, following injection of 10 mgm. of nicotine into a 9 kgm. dog with intact adrenals, whereas after excision of the adrenals, the same dose of nicotine only caused a rise from 24 mm. to about 30 mm. He concludes that practically the whole pressor effect of nicotine after destruction of the bulbo-spinal centres is due to the increase produced by it in the output of epinephrin. But he did not determine whether in the second part of his experiment a given dose of epinephrin would produce as great an effect as in the first part, and we know that great changes can occur in the magnitude of the response to a given quantity of epinephrin in the course of an experiment involving such an operation, even when the splanchnics have not been injured in the adrenalectomy. Five hundred cubic centimeters of Locke's solution had been injected to keep up the pressure before the first dose of nicotine was administered, but no mention is made of any solution having been injected after adrenalectomy although the pressure was still lower. We have seen that injection of Ringer's solution under conditions of low blood pressure may markedly augment the pressor effect of epinephrin, and no doubt this would be true also of vasomotor stimulation.

For example, 250 cc. of Ringer's solution was injected into a 9 kgm. dog when the blood pressure had sunk to 27 mm. of mercury. The heart's action improved and the blood pressure rose somewhat (to 50 mm. of mercury), and remained steady at about this level for a con-

siderable time. The effect on the blood pressure of 0.5 cc. of a 1:33,000 solution of adrenalin introduced into the jugular vein before the injection of the Ringer's solution was no greater than the effect of 0.5 cc. of a 1:130,000 after the injection. In another observation 0.5 cc. of 1:65,000 adrenalin before the injection of the Ringer's solution did not give as great an effect as 1:260,000 after the injection. As in all our papers, the concentration of adrenalin solutions is expressed in terms of the base, determined colorimetrically, by the method of Folin, Cannon and Denis (4).

Langley (5) obtained an excellent rise of pressure when nicotine was injected after the adrenals had been tied off from the circulation. In some of our own experiments, the nicotine injection was made while the adrenal blood was being collected, yet a good increase of pressure was seen. Here, of course, the bulbo-spinal centers may have participated in the action. And it may be assumed that if nicotine stimulates the ganglion cells on the path of the adrenal secretory fibres, or in the absence of ganglion cells on the path of these fibres (6) possibly some part of the medullary cells themselves, an increased epinephrin output may contribute to the rise of pressure. But all the evidence goes to show that even when the output has been proved by direct methods to be considerably augmented and the adrenal blood after being collected for a time is suddenly released the epinephrin factor in the increase of pressure is far subordinate to the vasomotor factor. And it would appear in the highest degree improbable that the excitation of the ganglion cells on the course of the vasomotors should be a negligible factor in the vasoconstriction in comparison with the excitation of the adrenal secretory fibres.

Cannon, Aub and Binger (7) conclude that "injection of nicotine in small amounts (3.5 to 7.5 mgm. in cats) results in augmented adrenal secretion." It is impossible, however, by the method used (collection of blood by a catheter from the inferior cava above the level of the adrenal veins) to arrive at any conclusion as to the effect of the drug upon the rate of output of epinephrin. For changes in the rate of the blood flow were not taken account of, and it has been already pointed out more than

once (2), although this would scarcely seem necessary, that to determine the output of epinephrin by applying blood to such objects as intestine strips or segments, two quantities must be measured, the concentration of epinephrin in the blood coming from the adrenals and the amount of blood passing through them in unit of time. That the concentration of epinephrin in the adrenal vein blood and, therefore, in the cava blood above the adrenal veins may be increased at some stage after the injection of such doses of nicotine is certain owing to the diminution in the rate of blood flow associated with the marked and prolonged fall of blood pressure succeeding the very brief rise. But such an increase of concentration is always observed when the blood flow in the cava is slowed from any cause whatever, provided that the epinephrin output continues unchanged or is diminished less than the rate of the blood flow.

Technique. Our experiments were all made upon cats. The technique has been described sufficiently in the first paper of this series (2). Direct determinations upon rabbit intestine (and uterus) segments of the epinephrin concentration in specimens of pure adrenal blood collected at a measured rate were chiefly relied upon. Corroborative evidence was obtained by a method of auto-assay, by means of the blood pressure reactions produced by adrenal blood when collected in a cava pocket for a given time and then released into the circulation.

Comparison of the (denervated) eye reactions in a normal cat with those in a cat whose epinephrin discharge had been interfered with by previous excision of one adrenal and denervation of the other, was also employed as a corroborative method, although it was recognized that the evidence afforded by this method was distinctly inferior to that yielded by the other two, especially the first mentioned, and far more difficult of interpretation.

One experiment was made to determine whether nicotine produced a demonstrable effect upon the epinephrin store of the adrenals.

EXPERIMENTS WITH INTRAVENOUS INJECTION OF NICOTINE AND ASSAY OF ADRENAL BLOOD ON RABBIT SEGMENTS

The experiments yielded strikingly clear and consistent results. The predominant action of nicotine upon the epinephrin output was shown to be a depressant or paralysing action. This

effect is easy to detect, begins early and lasts a relatively long time, its duration naturally depending upon the dose. Quantitatively the rate of output may be diminished, when the paralyzing action is at its height, to a third, a quarter or a fifth of the normal or initial output before the administration of the drug, or no epinephrin at all may be detected with certainty by the test objects. Gradually recovery ensues and the rate increases, but it may or may not (in these acute experiments) regain the original amount.

This stage of paralysis is preceded by a very brief period during which the epinephrin output is more or less markedly augmented, according to the dose and other conditions. Blood pressure tracings taken while the adrenal blood specimens were being collected, showed that the augmentation of the output coincided approximately with the period of increased blood pressure, and the diminished output with the much longer period during which the blood pressure remained below the original level. The diminution in the output was not due directly to the lowered blood pressure. For we have abundant evidence that with still lower pressures in the absence of nicotine, epinephrin continues to be liberated for long periods at the initial rate before the blood pressure was lowered. Also the abruptness with which the preliminary augmentation of the output gives place to the maximum diminution indicates that the initial stimulation has been quickly overborne by paralysis or depression just as in the case of the vasomotor mechanism.

It is to be presumed that both of these actions like the similar effects upon the efferent vasomotor paths are exerted upon sympathetic ganglion cells intercalated in the efferent secretory path of the adrenals (but see Elliott (6)).

With the smallest doses employed (about 0.1 mgm. of nicotine per kilogram of bodyweight) the depressant effect upon the epinephrin output was clearly demonstrated. None of the adrenal blood samples collected after these small doses of the poison, not even the samples taken immediately after its injection, corresponded to an output, calculated for the whole sample, greater than the original output before the nicotine injection. It was

shown, however, that this was simply due to the fact that the depressant action so quickly succeeded the stimulation that when the collection of the first sample after the nicotine lasted only one minute, the augmentation, which, of course, was less than with larger doses and continued only for a fraction of a minute, was totally masked by the diminished output during the rest of the minute.

In the first two experiments to be quoted (cats 281 and 283), although the dose was relatively large, we completely missed the transient preliminary augmentation of the output because we did not as yet know that it was so brief that it was necessary to cut down the interval between the nicotine injection and the collection of the adrenal blood to a minimum.

Condensed protocol. Cat 281; male; weight, 4.37 kgm.

Anesthetized with urethane. Obtained indifferent blood from the jugular. Made cava pocket. Collected adrenal blood.

11.45 a.m. First specimen, 2.1 grams in 30 seconds (4.2 grams per minute).

11.45½ a.m. Second specimen, 7.8 grams in 120 seconds (3.9 grams per minute).

11.54 a.m. Injected 2.5 mgm. nicotine intravenously.¹

11.56 a.m. Third adrenal specimen, 2.6 grams in 30 seconds (5.2 grams per minute).

11.56½ a.m. Fourth adrenal specimen, 7.0 grams in 120 seconds (3.5 grams per minute).

12.10 p.m. Fifth adrenal specimen, 1.9 grams in 30 seconds (3.8 grams per minute).

12.10½ a.m. Sixth adrenal specimen, 7.35 grams in 120 seconds (3.7 grams per minute).

12.15 p.m. Injected 5 mgms. nicotine intravenously.

12.17 p.m. Seventh adrenal specimen, 1.8 grams in 30 seconds (3.6 grams per minute).

12.17½ p.m. Eighth adrenal specimen, 4 grams in 150 seconds (1.6 grams per minute).

Obtained another specimen of venous blood. Combined weight of adrenals 0.553 gram.

¹ As in all of the experiments, the nicotine was injected in a 1:200 solution.

It is impossible to reproduce a sufficient number of tracings to illustrate adequately the epinephrin assays in any of the experiments. In figure 1 are given tracings showing the relative effects produced on a rabbit intestine segment by the three most important adrenal blood specimens. As the blood flows were nearly

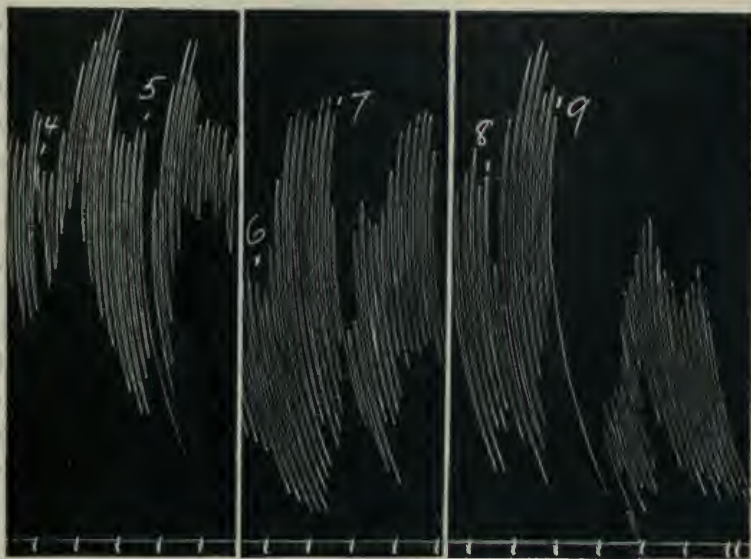


FIG. 1. INTESTINE TRACINGS. BLOODS FROM CAT 281

At 4, 6 and 8 Ringer was replaced by jugular blood and this at 5 by the fourth adrenal specimen (collected two and one-half minutes after nicotine injection); at 7 by the sixth adrenal specimen (collected sixteen and one-half minutes after injection of nicotine); at 9 by the second adrenal specimen (collected before injection of nicotine). All the bloods were diluted with three volumes Ringer.* (Reduced to two-thirds.)

* In all the intestine and uterus tracings time is marked in half minutes, in the blood pressure tracings in ten second intervals.

equal in these specimens, even the qualitative comparison of the tracings shows that nicotine produced a marked diminution in the epinephrin output. It was confirmed by uterus tracings that the sixth specimen was stronger than the fourth and the second stronger than the eighth. The detailed assay showed

that the second specimen, collected before injection of nicotine, was much stronger than 1:5,700,000 adrenalin, weaker than 1:3,000,000, not far from 1:4,300,000, probably slightly weaker. Taking it at 1:4,500,000, we get an output of 0.00087 mgm. per minute for the cat, or 0.0002 mgm. per kilogram per minute. The fourth specimen, whose collection was begun two and one-half minutes after the nicotine injection, was much weaker than the second (fig. 1, observations 5 and 9) although the blood flow was somewhat less in the fourth. Obviously, the output must have been greatly diminished at the time the fourth specimen was collected. The fourth specimen was found to be weaker than 1:11,400,000 adrenalin, somewhat weaker than 1:14,300,000. Taking it at 1:15,000,000, we get 0.00023 mgm. per minute for the cat, or 0.00005 mgm. per kilogram per minute, only one-fourth of the output before the administration of nicotine.

It must be remembered that if the transient preliminary increase in the output was present in this experiment, as is likely, seeing that we have never missed it, since we have known when to look for it, even with a dose relatively smaller than that used in this experiment, some of the epinephrin belonging to the period of excitation might have been included in the fourth specimen at the beginning of its collection. The reduction in the output during the greater part of the period of collection would then be much greater than that calculated from the assay. The sixth specimen (collected sixteen to seventeen minutes after nicotine) had a much smaller concentration of epinephrin than the second (fig. 1, observations 7 and 9), although the flow was slightly less. The depressant effect was, therefore, still quite marked, but some recovery had taken place since the sixth specimen was stronger than the fourth. It was demonstrated by three separate sets of observations (one set is reproduced in figure 1) that the sixth specimen was intermediate in concentration between the second and the fourth. It was the same whether the venous blood containing nicotine, collected at the end of the experiment, or the venous blood drawn before injection of nicotine, was employed as indifferent blood. In our assays of adrenal blood specimens collected after nicotine, we always test them

against nicotine indifferent blood as well as against nicotine-free indifferent blood. The assay showed that the sixth specimen was weaker than 1:7,100,000, weaker than 1:8,570,000, stronger than 1:11,400,000. It was finally taken at 1:9,500,000, corresponding to an output of 0.0004 mgm. per minute for the cat, or 0.00009 mgm. per kilogram per minute, nearly half the rate before the injection of nicotine. Accordingly, the recovery in the output sixteen or seventeen minutes after the injection of nicotine was substantial, but it was still much below the original output.

After the collection of the sixth specimen another larger dose of nicotine was administered. The eighth adrenal specimen was obtained two to three minutes thereafter. It was found to have about the same concentration of epinephrin as the second specimen, viz., 1:4,500,000. But as the blood flow during collection of the eighth was less than half as great as during collection of the second, the output per minute was much less, 0.00035 mgm. per minute for the cat, or 0.00008 mgm. per kilogram per minute. If the second injection of nicotine caused any excitation of the epinephrin secretion at this time, which we may assume from other experiments was the case, the effect was over before the eighth specimen was obtained, and only the depressant action was in evidence.

In the next experiment (cat 283) a much larger dose of nicotine was employed in the hope of obtaining the *increase* in epinephrin output which was the only action mentioned in the scanty literature of the subject.

Condensed protocol. Cat 283; male; weight, 3.38 kgm

Anesthetized with ether. Obtained indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

11.55 a.m. First specimen, 1.55 grams in 30 seconds (3 grams per minute).

11.55½ a.m. Second specimen, 5.55 grams in 120 seconds (2.8 grams per minute). Blood pressure at the end of collection of second specimen was 94 mm. mercury; (fig. 2, observation 3).

- 12.05 p.m. Injected 7.5 mgm. nicotine intravenously (fig. 2, observations 4 and 5); started artificial respiration (observation 6).
- 12.07 p.m. Third adrenal specimen, 0.7 gram in 60 seconds (0.7 gram per minute).
- 12.08 p.m. Fourth adrenal specimen, 2.55 grams in 420 seconds (0.37 gram per minute). Blood pressure during collection of third specimen was 34 mm. mercury (fig. 2, observation 7); blood pressure during collection of fourth specimen was 26 mm. mercury (fig. 2, observation 8).
- 12.25 p.m. Fifth adrenal specimen, 0.3 gram in 60 seconds (0.3 gram per minute).
- 12.26 p.m. Sixth adrenal specimen, 1.2 grams in 300 seconds (0.24 gram per minute). Blood pressure during collection of fifth specimen was 24 mm. mercury (fig. 2, observation 9); blood pressure during collection of sixth specimen was 22 mm. mercury (fig. 2, observation 10).

Obtained another specimen of venous blood. Combined weight of adrenals 0.33 gram.

There was, of course, in the blood pressure tracings of all the experiments a certain amount of lost time in making the marks, so that each should be moved a second or two to the left. It is still more important to remember that the signal marks, indicating the beginning or end of collection of the specimen, must all be shifted to the left in order to really correspond with the blood pressure curve, because of the dead space between the adrenal veins and the open end of the cannula. The number of seconds to be allowed for this will, of course, depend upon the rate of the blood flow. This correction cannot be exactly made since the dead space is not exactly known. In our experiments, it varied from one-half to one cc. according to the size of the cat and was somewhat less for the later than for the earlier specimens, since in putting in fresh cannulae, the cava pocket of course grew shorter.

Another factor which prevents an exact correspondence of the blood pressure curve with the curve of epinephrin output, is that although the latency of the adrenal response to stimulation of the secretory nerves, as shown in a previous paper (8) is exceedingly short, it cannot be supposed that epinephrin mobilized in response to stimulation of the secretory nerves will as quickly reach the blood as the arterioles will contract to vasomotor stimulation.

Figure 2 shows the points on the blood pressure tracing at which the various adrenal blood specimens were collected. Although the interval between the injection of the nicotine and the beginning of collection of the fourth specimen was only three minutes the period of increased blood pressure was long since over, and as we found out later, it was useless to look at this time for an increased epinephrin output.

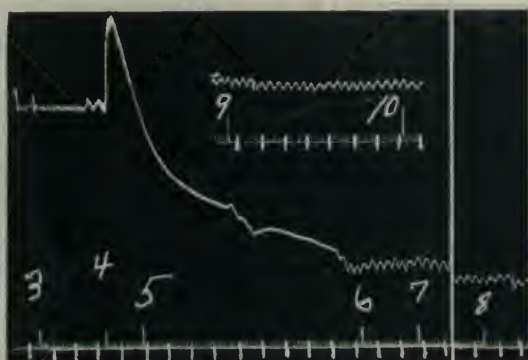


FIG. 2. BLOOD PRESSURE TRACING. CAT 283

3 marks the end of collection of the second adrenal specimen; 4 and 5 beginning and end of nicotine injection; at 6 artificial respiration was begun; 7 beginning of collection of third adrenal specimen; 8 end of collection of fourth adrenal specimen; 9 beginning of collection of fifth adrenal specimen; 10 end of collection of sixth adrenal specimen. Line of zero pressure same as time trace. (Reduced to two-thirds.)

The second specimen (taken before injection of nicotine) was found to be decidedly weaker than 1:5,300,000, and somewhat stronger than 1:8,000,000. It was taken at 1:7,000,000, corresponding to an output of 0.0004 mgm. per minute for the cat, or 0.00012 mgm. per kilogram per minute.

The fourth adrenal blood specimen (collected three minutes after the administration of nicotine) had a much greater concentration of epinephrin than the second (fig. 3). Since, however, the blood flow during collection of the fourth specimen was only one-eighth as great as during collection of the second, the

mere qualitative comparison of the inhibitory effects produced on the intestine segment yields no information whatever as to any change in the rate of output. As a matter of fact, the assay proved that the fourth specimen, (which was much weaker than 1:1,000,000, weaker than 1:1,300,000, weaker than 1:2,000,000, stronger than 1:2,700,000 and was finally taken at 1:2,400,000), gave an output of only 0.00015 mgm. per minute for the cat, or

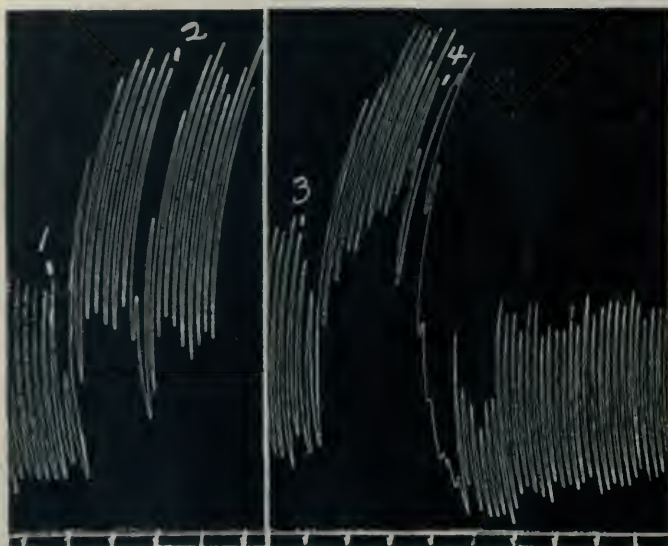


FIG. 3. INTESTINE TRACINGS. BLOOD FROM CAT 283

At 1 and 3 Ringer was replaced by jugular blood and this at 2 by the second adrenal specimen (collected before injection of nicotine); at 4 by the fourth adrenal specimen (collected three minutes after injection of nicotine). All the bloods were diluted with three volumes Ringer. (Reduced to two-thirds.)

0.000045 mgm. per kilogram per minute, only about one-third of the output before nicotine. This was not because the blood flow was so small that the concentration of epinephrin had reached the possible maximum. It was far below the normal maximum, and, indeed, it will be shown presently that one of the characteristics of the nicotine stimulation of the epinephrin secretion, which distinguishes it from the stimulation due to strychnine,

nine, is that the possible normal maximum concentration can be much exceeded. The depressant effect of the nicotine was, therefore, distinctly in evidence at this time, not only as regards the vasomotor, but as regards the adrenal secretory fibres.

The sixth adrenal specimen (taken twenty-one minutes after nicotine) was found to be much weaker than 1:1,300,000, weaker than 1:2,000,000, and approximately equal to 1:2,700,000, corresponding to an output of 0.00009 mgm. per minute for the cat, or 0.000025 mgm. per kilogram per minute, little more than one-fifth of the output before nicotine. Figure 4 shows that the sixth specimen was somewhat weaker than the fourth.

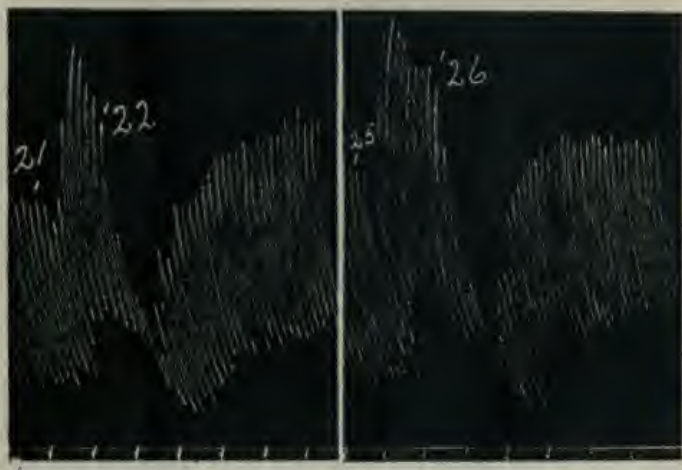


FIG. 4. INTESTINE TRACINGS. BLOOD FROM CAT 283

At 21 and 25 Ringer was replaced by venous blood collected after the injection of nicotine; and this at 22 by the fourth adrenal specimen (collected three minutes after injection of nicotine); at 26 by the sixth adrenal specimen (collected twenty minutes after the injection of nicotine). All the bloods were diluted with three volumes Ringer. (Reduced to two-thirds.)

Condensed protocol. Cat 284; male; weight, 2.85 kgm.

Anesthetized with ether. Obtained indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

11.17 a.m. First specimen, 1.2 grams in 30 seconds (2.4 grams per minute).

- 11.17½ a.m. Second specimen, 4.0 grams in 120 seconds (2 grams per minute). Blood pressure 100 mm. mercury (fig. 5, observation 1).
- 11.25 a.m. Injected 2 mgn. nicotine intravenously (fig. 5, observations 2 and 3).
- 11.25½ a.m. Third adrenal specimen, 0.9 gram in 30 seconds (1.8 grams per minute).
- 11.26 a.m. Fourth adrenal specimen, 1.6 grams in 120 seconds (0.8 gram per minute). Blood pressure at beginning of collection of third specimen was 114 mm. mercury (fig. 5, observation 4); blood pressure during collection of fourth specimen was falling. The pressure at the beginning of the collection was 52 mm. and at the end of the collection was 20 mm. mercury (fig. 5, observation 5).
- 11.40 a.m. Fifth adrenal specimen, 0.75 gram in 30 seconds (1.5 grams per minute).
- 11.40½ a.m. Sixth adrenal specimen, 3.9 grams in 180 seconds (1.3 grams per minute). Blood pressure during collection of fifth specimen was 70 mm. mercury (fig. 5, observation 8); blood pressure during collection of sixth specimen was 66 mm. mercury (fig. 5, observation 9).

Obtained another specimen of venous blood. Combined weight of adrenals 0.502 gram.

In the above experiment (cat 284) adrenal blood was collected almost immediately after completion of the injection of a much smaller dose of nicotine than in the last experiment, with the object of catching, if possible, a sample which would show an increased output before the paralysis came on. The expectation was realised. Figure 5 shows the position on the blood pressure curve of the various adrenal specimens. The third adrenal specimen (collected about one-half minute after the nicotine injection) had by far the greatest concentration of epinephrin of all the specimens (fig. 6, observation 34). It was proved by the assay to be much stronger than 1:660,000 adrenalin, stronger than 1:330,000 adrenalin (fig. 6, observations 30 and 36). Several other observations, not reproduced, showed that it was fully as strong as 1:300,000. But taking it at 1:300,000, we get for the

output of epinephrin, at this time, 0.006 mgm. per minute for the cat, or 0.002 mgm. per kilogram per minute. In as much as the time at which collection of a specimen begins nominally must be corrected for the time required to fill up the dead space in the cava and cannula at the given rate of flow, the blood of the third specimen really began to leave the adrenals a very few seconds after injection of the nicotine.

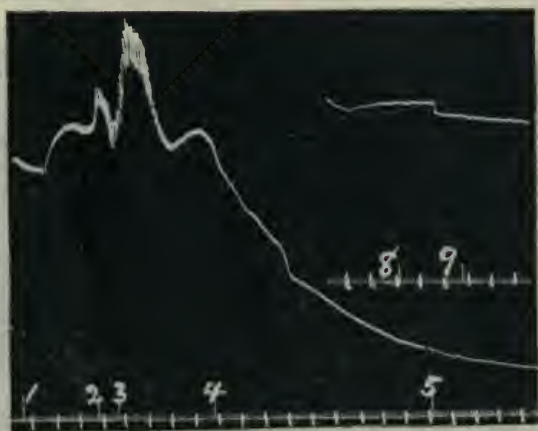


FIG. 5. BLOOD PRESSURE TRACING. CAT 284

1, blood pressure during collection of adrenal blood before nicotine injection; 2 to 3, injection of nicotine; 4, beginning of collection of third adrenal specimen; 5, during collection of the fourth adrenal specimen; 8, beginning of collection of fifth adrenal specimen; 9, during collection of sixth adrenal specimen. Line of zero pressure corresponds with the time trace and is moved up 15 mm. and the figure then reduced to two-thirds.

The second adrenal blood specimen (collected before injection of nicotine) was assayed at 1:7,000,000, corresponding to an output of 0.0003 mgm. per minute for the animal, or 0.0001 mgm. per kilogram per minute. The third specimen, therefore, represents a rate of output not less than twenty times the original output before nicotine. The sixth adrenal specimen (collected a quarter of an hour after the administration of nicotine when the blood pressure had recovered to 100 mm. of mercury), had a somewhat greater concentration of epinephrin than the second

specimen (fig. 7, observations 2 and 6). It was assayed at 1:6,000,000, corresponding to an output of 0.0002 mgm. per minute for the cat, or 0.00007 mgm. per kilogram per minute. The initial output had not been quite recovered at this time.

The fourth adrenal blood specimen (collected one minute to three minutes after the nicotine injection, while the blood pressure was falling to its minimum level) was found to be much stronger than either the sixth or the second (fig. 7). The blood flow, of

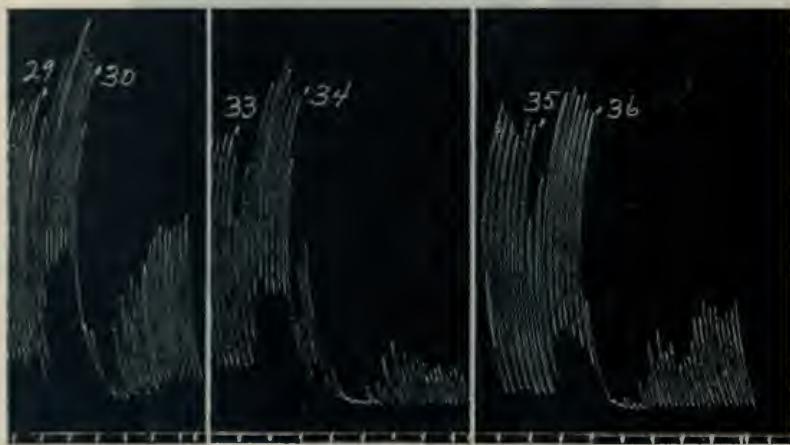


FIG. 6. INTESTINE TRACINGS. BLOODS FROM CAT 284

At 29, 33 and 35 Ringer was replaced by venous blood collected after injection of nicotine; and this at 30 by venous blood to which was added adrenalin to make a concentration of 1:660,000; at 34 by the third adrenal specimen (collected immediately after injection of nicotine); at 36 by venous blood to which was added adrenalin to make a concentration of 1:330,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

course, owing to the low blood pressure was much less. The inhibition of the intestine segment produced by the fourth specimen was decidedly less than that caused by the third. The difference was greater than would appear from comparison of figure 8, observation 24, and figure 6, observation 34, since the sensitiveness of the segment to epinephrin was proved to have diminished between observations 26 and 28 (not reproduced).

The assay showed that the fourth specimen was stronger than 1:930,000 (fig. 8, observations 24 and 26), and somewhat stronger than 1:660,000. Taking it at 1:600,000, we get 0.0013 mgm. per minute for the cat, or 0.00045 mgm. per kilogram per minute; four or five times the rate of the output before nicotine. It is practically certain that this relatively high output is due entirely to an overlapping of the period of excitation, which reached its maximum during collection of the third specimen, into the first part of the period of collection of the fourth. If the average rate of output for the third specimen continued for the first half

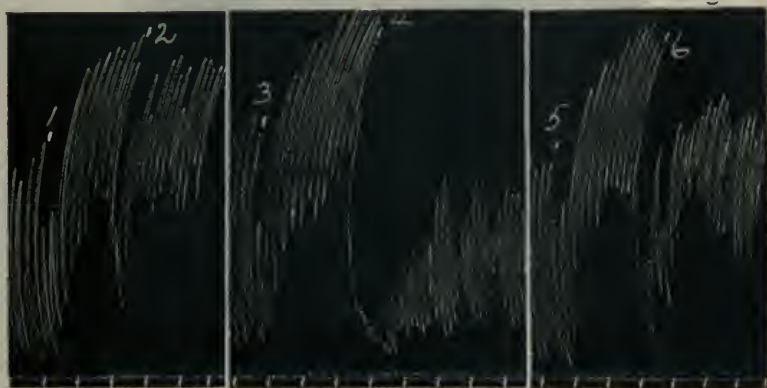


FIG. 7. INTESTINE TRACINGS. BLOODS FROM CAT 284

At 1, 3 and 5 Ringer was replaced by jugular blood and this at 2 by the second adrenal specimen (collected before injection of nicotine); at 4 by the fourth adrenal specimen (collected one minute after injection of nicotine); at 6 by the sixth adrenal specimen (collected fifteen minutes after injection of nicotine). All the bloods were diluted with three volumes Ringer. (Reduced to one-half.)

minute of collection of the fourth, this would account for the whole content of epinephrin in the fourth specimen even if none at all was liberated during the remaining one and one-half minutes. It must be remembered that owing to the "lag" entailed by the filling of the dead space, the real commencement of collection of the fourth specimen would be only a little more than one-half minute after the nicotine injection. From the evidence of other experiments it is almost certain that the paralysing

action of the drug would be fully developed during the latter part of the period of collection of the fourth specimen and would continue till it began to disappear as the pressure gradually rose.

The proportion of serum in the blood, determined by the hematocrite (after 34 minutes rotation at 4000 times a minute) was 52 per cent. The epinephrin concentration in the serum of the third specimen must, therefore, have been nearly 1:150,000. This is fully three times the possible normal maximum concen-

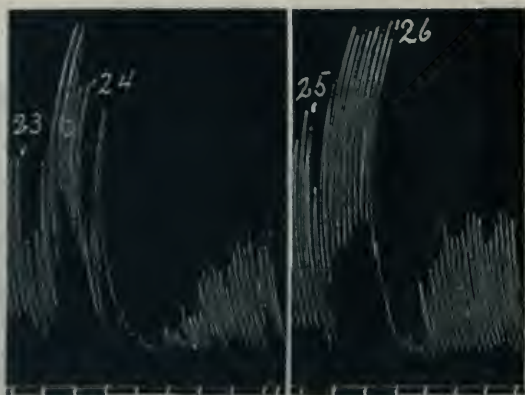


FIG. 8. INTESTINE TRACINGS. BLOODS FROM CAT 284

At 23 and 25 Ringer was replaced by venous blood (collected after injection of nicotine) and this at 24 by the fourth adrenal specimen (collected one minute after injection of nicotine); at 26 by venous blood to which was added adrenalin to make a concentration of 1:1,000,000. All the bloods were diluted with three volumes Ringer (the adrenalin blood after adding the adrenalin). (Reduced to one-half.)

tration as defined in the first paper of this series (2). It was there stated that in none of the strychnine experiments had the possible normal maximum concentration of epinephrin (about 1:500,000, as determined on rabbit segments) been exceeded even with the slowest flows and the greatest increases in the output. Apparently, then, the transient stimulation of the secretion caused by nicotine differs from the long lasting stimulation caused by strychnine, in that the former for the brief period for which it lasts can force up the normal maximum con-

centration while the latter cannot. If this distinction is well founded it is of considerable interest. For it indicates that the strychnine action is essentially a speeding up of the normal process presumably by an increase in the excitability of the central mechanism, whereas the nicotine action is an artificial and quite unphysiological stimulation of the sympathetic relay on the efferent path.

Condensed protocol. Cat 285, male, weight, 4.4 kgm.

Anesthetized with ether. Obtained indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

- 11.40 a.m. First specimen, 2.6 grams in 30 seconds (5.2 grams per minute). Second specimen, 9.2 grams in 120 seconds (4.6 grams per minute). Blood pressure 146 mm. mercury (fig. 9, observation 3).
- 11.50 a.m. Injected 2 mgm. nicotine intravenously (fig. 9. observations 4 and 5).
- 11.50½ a.m. Third adrenal specimen, 1.35 grams in 30 seconds (2.7 grams per minute).
- 11.51 a.m. Fourth adrenal specimen, 2.05 grams in 120 seconds (1.03 grams per minute).
- 11.53 a.m. Fifth adrenal specimen, 3.35 grams in 360 seconds (0.56 gram per minute).

Blood pressure at the beginning of the collection of the third specimen was 116 mm. mercury (fig. 9. observation 6); at the beginning of the collection of the fourth specimen 70 mm. mercury (observation 7); at the beginning of the collection of the fifth specimen 27 mm. mercury (observation 8).

- 12.12 p.m. Sixth adrenal specimen, 1.8 grams in 30 seconds (3.6 grams per minute).
- 12.12½ p.m. Seventh adrenal specimen, 9.3 grams in 180 seconds (3.1 grams per minute).

Blood pressure during collection of the sixth specimen was 92 mm. mercury (fig. 9. observation 16); blood pressure during collection of the seventh specimen was 85 mm. mercury (fig. 9, observation 17).

Obtained another specimen of venous blood. Combined weight of adrenals 0.553 gram.

In the above experiment (cat 285) a still smaller dose of nicotine, reckoned on the bodyweight, was employed in order to see whether the stage of depression which cuts short the preliminary stimulation could thus be postponed, permitting a longer period of increased output. This was not found to be the case. In essentials the results were precisely the same as in the preceding experiment. The blood pressure curve followed exactly the same course and the changes in epinephrin output were roughly parallel to the blood pressure curve.

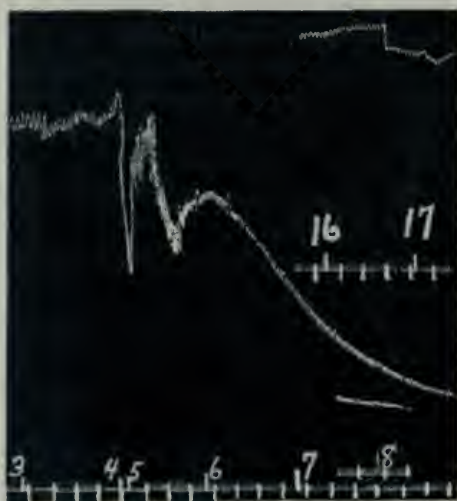


FIG. 9. BLOOD PRESSURE TRACING. CAT 285

3, Blood pressure during collection of second adrenal specimen (before nicotine injection); 4 to 5, injection of nicotine; 6, beginning of collection of third adrenal specimen; 7, beginning of collection of fourth adrenal specimen; 8, beginning of collection of fifth adrenal specimen; 16, end of collection of sixth adrenal specimen; 17, end of collection of seventh adrenal specimen. Line of zero pressure corresponds with the time trace. (Reduced to two-thirds.)

Figure 9 shows the points on the blood pressure curve at which the various adrenal blood specimens were collected. The second specimen, collected before injection of nicotine, produced a much smaller inhibition of the intestine segment than the fourth speci-

men, collected one minute after nicotine (fig. 10). Since the blood flow during collection of the fourth specimen was less than one-fourth of the flow during collection of the second, this difference would not of itself prove that the epinephrin output was increased for the fourth specimen. The assay on rabbit intestine (and uterus) segments showed, however, that this was the case.

The second specimen was found to be much weaker than 1:2,600,000 adrenalin, weaker than 1:4,000,000, weaker than 1:5,200,000, decidedly stronger than 1:8,000,000, somewhat

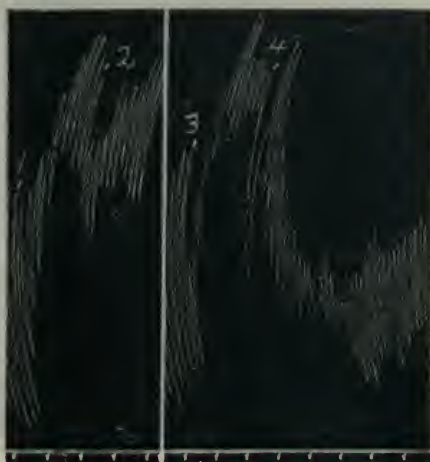


FIG. 10. INTESTINE TRACINGS. BLOODS FROM CAT 285

At 1 and 3 Ringer was replaced by indifferent (jugular) blood and this at 2 by the second adrenal specimen (collected before injection of nicotine); at 4 by the fourth adrenal specimen (collected one minute after injection of nicotine). All the bloods were diluted with three volumes Ringer. (Reduced to one-half.)

stronger than 1:6,600,000. It was finally assayed at 1:6,500,000 corresponding to an output of 0.0007 mgm. per minute for the cat, or 0.00016 mgm. per kilogram per minute.

Collection of the third specimen was begun nominally thirty seconds after the nicotine injection, but allowing for the filling of the dead space in the cava and cannula, the first blood included in the specimen must have left the adrenals not later than fifteen seconds after the injection. The third specimen produced an

inhibitory effect on the intestine segment decidedly greater than that produced by the fourth specimen (fig. 11, observations 28 and 26), although the blood flow was nearly three times as great in the case of the third specimen.

The assay proved that the third specimen had an epinephrin concentration much greater than 1:1,000,000, greater than 1:330,000, less than 1:220,000 (intestine tracings not repro-

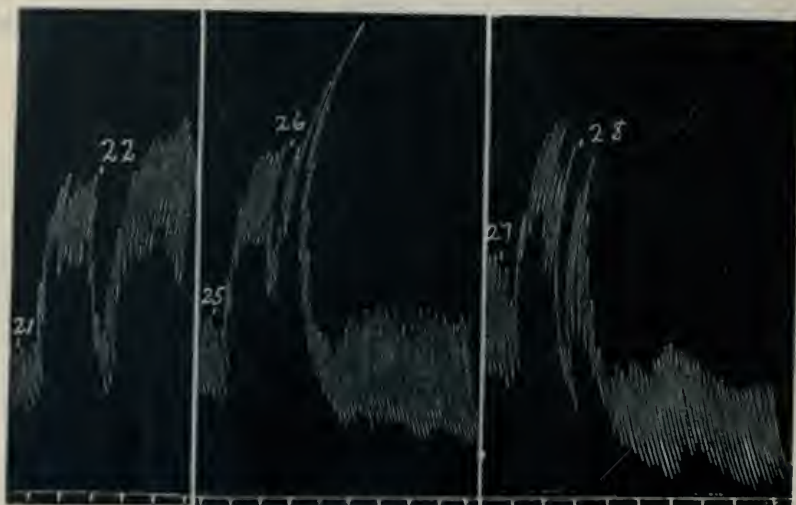


FIG. 11. INTESTINE TRACINGS. BLOODS FROM CAT 285

At 21, 25 and 27 Ringer was replaced by venous blood collected after injection of nicotine; and this at 22 by the seventh adrenal specimen (collected twenty-two minutes after injection of nicotine); at 26 by the fourth adrenal specimen (collected one minute after injection of nicotine); at 28 by the third adrenal specimen (collected immediately after injection of nicotine). All the bloods were diluted with three volumes Ringer. (Reduced to one-half.)

duced, confirmed by uterus, figure 12, observations 76 and 77). It was taken at 1:275,000, corresponding to an output of 0.01 mgm. per minute for the cat, or 0.0023 mgm. per kilogram per minute, fourteen times the initial rate of output.

The average output for the period of collection of the fourth specimen was not nearly one-third of that during the collection of the third specimen. No doubt towards the end of the col-

lection of the fourth specimen it was much less, since the output for the fifth specimen, immediately succeeding the fourth, was no more than half the initial output (0.00035 mgm. per minute for the cat, or 0.0008 mgm. per kilogram per minute).

The seventh specimen, collected twenty-two and one-half minutes after injection of nicotine, when the blood pressure had again risen considerably caused a much smaller inhibition of the intestine segment than either the third or the fourth specimens

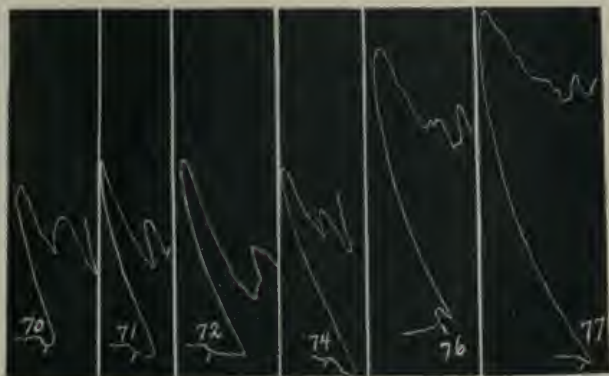


FIG. 12. UTERUS TRACINGS. BLOODS FROM CAT 285

At 70 Ringer was replaced by jugular blood to which was added adrenalin to make a concentration of 1:6,600,000; at 71 by the second adrenal specimen (collected before injection of nicotine); at 72 by the seventh adrenal specimen (collected twenty-two minutes after injection of nicotine); at 74 by jugular blood to which was added adrenalin to make a concentration of 1:6,600,000 and also sufficient nicotine to make 0.01 mgm. per cubic centimeter of blood; at 76 by the third adrenal specimen; at 77 by jugular blood to which was added adrenalin to make a concentration of 1:220,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

(fig. 11), and a somewhat smaller effect than the second specimen (intestine tracings not reproduced, but confirmed by uterus tracings, figure 12, observations 71 and 72, and by other uterus observations). It was assayed at 1:7,000,000 epinephrin, equivalent to an output of 0.00045 mgm. per minute for the cat, or 0.0001 mgm. per kilogram per minute. Even at this time the original output before nicotine had not yet been regained. The

proportion of serum in the blood was 56.5 per cent as determined by the electrical conductivity method. The hematocrite gave 46 per cent after fifteen minutes rotation; 49.5 per cent after seven minutes more and 52 per cent after a further twelve minutes. With 56 per cent of serum the concentration of epinephrin in the third specimen must have been 1: 150,000, a concentration much greater than the normal maximum.

In the next experiment (cat 286) the dose of nicotine was again reduced (to 0.25 mgm. per kilogram) but the depressant effect upon the epinephrin output was as prominent and the preliminary stimulating effect as fleeting as in any of the experiments with larger doses. The depressant effect, indeed, came out more sharply in specimens of adrenal blood collected soon after the nicotine injection than with the larger doses, doubtless because the smaller increase in the output produced in the first stage did not mask the depression in the succeeding specimens by overlapping.

Condensed protocol. Cat 286, male, weight, 4.0 kgm.

Anesthetized with ether. Obtained indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

11.45 a.m. First specimen, 2.5 grams in 30 seconds (5 grams per minute). Second specimen, 7.1 grams in 90 seconds (4.7 grams per minute). Blood pressure during collection of second specimen was 150 mm. mercury (fig. 13, observation 2).

11.48 a.m. Injected 1 mgm. nicotine intravenously (fig. 13, observation 4).

11.48½ a.m. Third adrenal specimen, 1.7 grams in 30 seconds (3.4 grams per minute).

11.49 a.m. Fourth adrenal specimen, 4.15 grams in 90 seconds (3.1 grams per minute).

11.50½ a.m. Fifth adrenal specimen, 5.2 grams in 90 seconds (3.4 grams per minute).

Blood pressure at the beginning of the collection of the third specimen was 130 mm. mercury; (fig. 13, observation 5) at the beginning of the collection of the fourth specimen 108 mm. mercury (observation 6); at the end of the collection of the fifth, 122 mm. mercury, (observation

7). The respiration was good throughout the experiment; artificial respiration was not needed.

12.03 p.m. Sixth adrenal specimen, 2.6 grams in 30 seconds (5.2 grams per minute).

12.03½ p.m. Seventh adrenal specimen, 9.15 grams in 120 seconds (4.6 grams per minute).

12.05½ p.m. Eighth adrenal specimen, 6.5 grams in 90 seconds (4.3 grams per minute).

Blood pressure at the beginning of the collection of the sixth specimen was 146 mm. mercury; (fig. 13, observation 9); at the beginning of

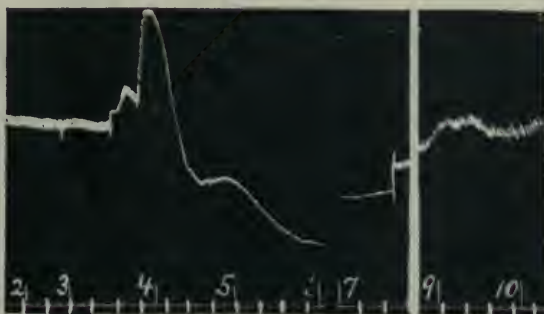


FIG. 13. BLOOD PRESSURE TRACING. CAT 286

2, Blood pressure during collection of second adrenal specimen; 3, end of collection of second specimen; 4, end of injection of 1 mgm. nicotine; 5, beginning of collection of third adrenal specimen; 6, beginning of collection of fourth adrenal specimen; 7, end of collection of fifth adrenal specimen; 9, beginning of collection of sixth adrenal specimen; 10, beginning of collection of seventh adrenal specimen. Zero line corresponds with time trace and is moved up 40 mm. and the figure then reduced to two-thirds.

collection of the seventh specimen 148 mm. mercury (observation 10); and during the collection of the eighth specimen, 148 mm. mercury.

Obtained another specimen of venous blood. Combined weight of adrenals 0.372 gram.

Figure 13 indicates the points on the blood pressure curve at which the adrenal blood specimens were collected. Figure 14 shows that the third adrenal specimen, collected half a minute, or allowing for the dead space, not much more than fifteen seconds, after the nicotine injection, had a much greater concen-

tration of epinephrin than the fourth specimen, collected immediately thereafter, which indeed produced little if any inhibition of the intestine segment. Since the blood flow for the fourth was practically the same as for the third, even a little less, this of itself indicates quite clearly that the output during collection of the third specimen must have been greater than during collection of the fourth. The eighth specimen (collected seventeen and one-half minutes after injection of nicotine) was also decidedly stronger than the fourth, although much weaker than the third.

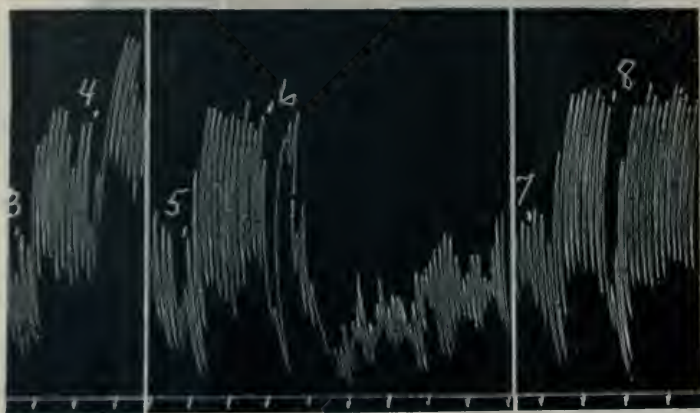


FIG. 14. INTESTINE TRACINGS. BLOODS FROM CAT 286

At 3, 5 and 7 Ringer was replaced by jugular blood and this at 4 by the fourth adrenal specimen (collected one minute after injection of nicotine); at 6 by the third adrenal specimen (collected immediately after injection of nicotine); at 8 by the eighth adrenal specimen (collected seventeen minutes after nicotine injection). All the bloods were diluted with three volumes Ringer. (Reduced to two-thirds.)

In other observations, e.g., observations 40 and 42 in figure 15, it was demonstrated that neither the fourth nor the fifth adrenal blood specimens caused any inhibition of the intestine segment, whereas the eighth specimen always gave a fair inhibition (fig. 15, observation 46). The eighth specimen did not differ much in concentration from the second specimen, collected before injection of nicotine (fig. 16), but was found by the assay

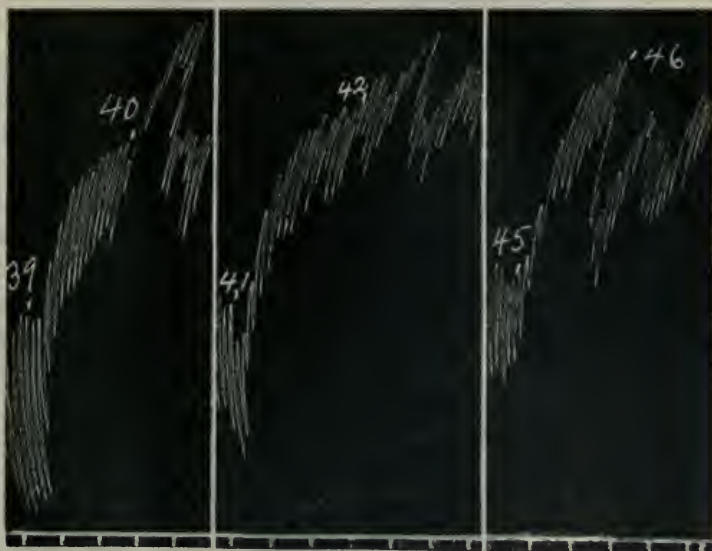


FIG. 15. INTESTINE TRACINGS. BLOODS FROM CAT 286

At 39, 41 and 45 Ringer was replaced by venous blood collected after injection of nicotine; and this at 40 by the fourth adrenal specimen (collected one minute after injection of nicotine); at 42 by the fifth adrenal specimen (collected two and one-half minutes after injection of nicotine); at 46 by the eighth adrenal specimen (collected seventeen minutes after injection of nicotine). All the bloods were diluted with three volumes Ringer. (Reduced to two-thirds.)

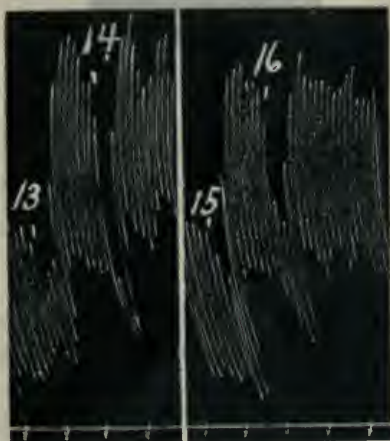


FIG. 16. INTESTINE TRACINGS. BLOODS FROM CAT 286

At 13 and 15 Ringer was replaced by jugular blood, and this at 14 by the eighth adrenal specimen (collected seventeen minutes after injection of nicotine); at 16 by the second adrenal specimen (collected before injection of nicotine). The bloods were diluted with three volumes Ringer. (Reduced to two-thirds.)

to be slightly weaker. It was confirmed by observations on the uterus that the fourth specimen gave practically no effect and certainly no greater effect than indifferent (jugular) blood, while the third specimen caused a very great increase of tone. Indifferent blood made up with adrenalin to a concentration of 1:7,000,000 produced a far greater reaction than the fourth specimen, although far less than the third (fig. 17).

The detailed assay showed that the second specimen was much stronger than 1:10,600,000 adrenalin, stronger than 1:8,000,000,

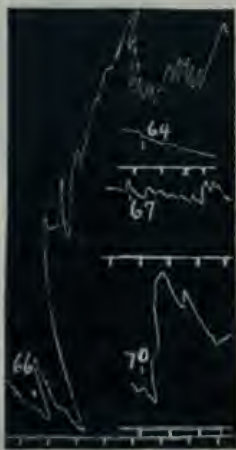


FIG. 17. UTERUS TRACINGS. BLOODS FROM CAT 286

At 64 and 67 Ringer was replaced by the fourth adrenal specimen (collected one minute after injection of nicotine); at 66 by the third adrenal specimen (collected immediately after injection of nicotine); at 70 by jugular blood to which was added adrenalin to make a concentration of 1:7,000,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin. (Reduced to one-half.)

weaker than 1:5,300,000, and approximately equal to 1:6,500,000, equivalent to an output of epinephrin of 0.00072 mgm. per minute for the cat, or 0.00018 mgm. per kilogram per minute.

The third specimen was found to be much stronger than 1:1,300,000, somewhat stronger than 1:530,000, but decidedly weaker than 1:330,000. It was assayed at 1:500,000, corresponding to an epinephrin output of 0.0068 mgm. per minute for

the cat, or 0.0017 mgm. per kilogram per minute, more than nine times the output before the injection of nicotine. The percentage of serum in the blood was 67, as determined by the electrical method; by the hematocrite 57 with fifteen minutes, 60 with twenty-two minutes, and 62 with thirty minutes rotation. The concentration of epinephrin in the serum of the third specimen would, therefore, be about 1:330,000.

As already stated, the fourth specimen, collected immediately after the third, and the fifth specimen, collected immediately after the fourth, did not contain an amount of epinephrin detectable by the segments worked with, so that if any epinephrin at all was present in these specimens the output during their collection must have been much less than the original output. A period of marked depression lasting for several minutes must, therefore, have succeeded the intense brief stimulation during collection of the third specimen. It is practically certain that this period of stimulation was entirely over before the end of collection of that specimen, else the fourth specimen would necessarily have been overlapped and must have contained epinephrin in detectable amount. It is, accordingly improbable that the stimulation in this experiment lasted more than a half minute, if so long.

The eighth specimen was shown to be weaker than 1:5,700,000 adrenalin, stronger than 1:6,660,000, somewhat stronger than 1:6,000,000. It was taken at 1:5,850,000, corresponding to an output of 0.00073 mgm. per minute for the cat, or 0.00018 mgm. per kilogram per minute, the same as in the second specimen, taken before nicotine injection. Recovery from the depressant effect was therefore complete at this time, seventeen and one-half minutes after the administration of the drug.

The seventh specimen, collected just before the eighth, had a smaller concentration, but in the absence of an exact assay of this specimen, it is not possible to know whether the output was smaller, as the flow for the seventh was somewhat larger than for the eighth.

The next experiment (cat 298) was performed with the object of assaying by the colorimetric method of Folin, Cannon and

Denis (4) the adrenal blood specimen taken immediately after the injection of nicotine. It was argued that with concentrations of epinephrin as great as had been observed previously during the brief preliminary period of stimulation by the drug a definite colorimetric reaction ought to be obtained. This would, of course, constitute the best possible corroboration of the results deduced from the assays on the segments. The colorimetric method is not delicate enough to be used with the epinephrin concentrations ordinarily present in adrenal blood.

Condensed protocol. Cat 298, female, weight 3.35 kgm.

Anesthetized with ether. Obtained indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

11.03 a.m. First specimen, 1.7 grams in 45 seconds (2.2 grams per minute).

11.03 $\frac{3}{4}$ a.m. Second specimen, 5.35 grams in 180 seconds (1.8 grams per minute). Blood pressure during collection of second specimen was 80 mm. mercury (fig. 18, observation 4).

11.10 $\frac{1}{2}$ a.m. Injected 2 mgm. nicotine intravenously (fig. 18, observation 5).

11.11 a.m. Third adrenal specimen, 2.4 grams in 60 seconds (2.4 grams per minute).

11.12 a.m. Fourth adrenal specimen, 5.55 grams in 360 seconds (0.92 gram per minute).

Blood pressure at the beginning of the collection of the third specimen was 110 mm. mercury (fig. 18, observation 6); at the beginning of the collection of the fourth specimen 60 mm. mercury (observation 7).

11.23 a.m. Obtained another specimen of indifferent blood from jugular (nicotine blood).

11.28 $\frac{1}{2}$ a.m. Fifth adrenal specimen, 1.7 grams in 45 seconds (2.2 grams per minute).

11.29 $\frac{1}{4}$ a.m. Sixth adrenal specimen, 5.6 grams in 180 seconds (1.9 grams per minute).

Blood pressure at the beginning of the collection of the fifth specimen was 76 mm. mercury; at the beginning of the collection of the sixth specimen 78 mm. mercury (fig. 18, observation 10).

Obtained another specimen of venous blood. Combined weight of adrenals 0.418 gram.

The expectation was realized. A dose of nicotine was chosen which seemed likely to be large enough to give a strong effect, but yet not so large as to cut short the stimulation almost instantaneously by the succeeding paralysis. As in a cat it was not possible to obtain enough blood in a half minute collection to permit a complete assay on the segments as well as the colorimetric assay, it was decided to limit the segment assay for the third specimen to determining a concentration of adrenalin which was clearly less than that of the specimen. The other specimens

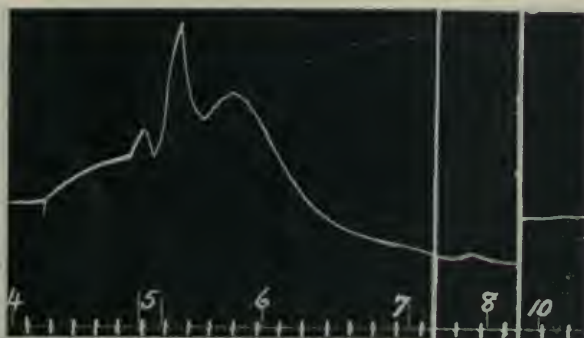


FIG. 18. BLOOD PRESSURE TRACING. CAT 298

4, End of collection of second adrenal specimen; 5, injection of nicotine; 6, beginning of collection of third adrenal specimen; 7, beginning of collection of fourth adrenal specimen; 8, end of collection of fourth adrenal specimen; 10, beginning of collection of sixth adrenal specimen. Zero line corresponds with time trace and is moved up 15 mm. and the figure then reduced to two-thirds.

were carefully assayed as usual by the segment reactions. Figure 18 shows the points on the blood pressure tracing at which the adrenal specimens were collected.

The second adrenal blood specimen, collected before injection of nicotine, was found to be decidedly stronger than 1:4,000,000 adrenalin, much weaker than 1:1,300,000, weaker than 1:2-660,000 (confirmed by several observations). It was taken at 1:3,000,000, corresponding to an output of epinephrin of 0.0006 mgm. per minute for the cat, or 0.00018 mgm. per kilogram per minute.

The third adrenal specimen, the collection of which was begun half a minute after the nicotine injection (allowing for the dead space within fifteen to twenty seconds after the injection), gave an enormously greater inhibition with the intestine segment than any of the other specimens (fig. 19, observation 27). It was shown by the uterus segment that the blood was certainly not weaker than 1:330,000 epinephrin. The serum was approximately assayed by the colorimetric method at 1: 300,000. The blood was very rich in serum (over 80 per cent). Blood with a

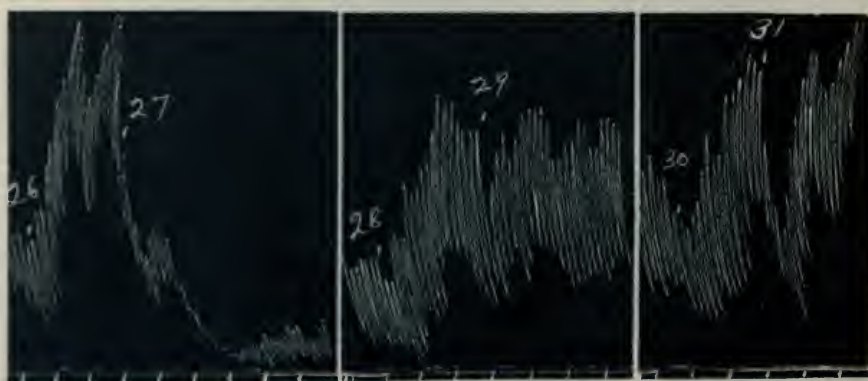


FIG. 19. INTESTINE TRACINGS. BLOODS FROM CAT 298

At 26, 28 and 30 Ringer was replaced by jugular blood obtained after nicotine injection; and this at 27 by the third adrenal specimen (collected immediately after the nicotine injection); at 29 by the fourth adrenal specimen (collected one and one-half minutes after nicotine injection); at 31 by the sixth adrenal specimen (collected nineteen minutes after nicotine injection). All the bloods were diluted with three volumes Ringer. (Reduced to four-sevenths.)

concentration of 1:330,000 would correspond to serum with a concentration of 1:265,000. The epinephrin output, taking the concentration of the third specimen at 1:330,000 would be 0.0073 mgm. per minute for the cat, or 0.0022 mgm. per kilogram per minute, more than twelve times the initial output before nicotine.

The fourth adrenal specimen, taken immediately after the third, gave hardly any reaction with the intestine segment (fig. 19, observation 29, no inhibition at all in another observation),

although the blood flow during its collection was not much more than one-third of that for the third specimen. It was shown that the output at the time the fourth specimen was taken (beginning one and one-half minutes after the nicotine injection, or about one and one-quarter minutes allowing for lag due to the dead space) must have been much less than 0.00006 mgm. per kilogram per minute, since its concentration was much less than 1:4,000,000 epinephrin, if any at all was present. In other words, the rate of output at the time of collection of the fourth specimen was much less than one-fortieth of the rate for the third. This indicates clearly that the period of excitation was completely over before the end of collection of the third specimen, i.e. (allowing for the dead space), at most seventy-five seconds after the administration of nicotine. As it is quite improbable that the beginning of the fourth specimen coincided exactly with the end of the period of excitation, the duration of the latter may be assumed to have been less than a minute and a quarter. If only half of the third specimen passed through the adrenals during the period of excitation the concentration of epinephrin in this portion of the blood must have been twice the concentration calculated on the whole specimen, and the increase in the rate of output as compared with the output before nicotine double the calculated increase for the whole specimen.

The sixth adrenal specimen, collected about nineteen minutes after the injection of nicotine, gave a much greater reaction with the intestine segment than the fourth (fig. 19, observation 31), although the blood flow was twice as great. It was assayed at 1:4,000,000 epinephrin, corresponding to an output of 0.00047 mgm. per minute for the cat, or 0.00014 mgm. per kilogram per minute. In the eleven minutes between the end of collection of the fourth and the beginning of collection of the sixth specimen the output had accordingly recovered considerably, although it was not yet quite equal to the original output before nicotine.

Two experiments were made with much smaller doses than in any of the preceding experiments, in order to see whether the smaller doses might not bring out more clearly the stimulation

effect in comparison with the depression of the output. One of the animals received 0.1 mgm. per kilogram of bodyweight and the other 0.08 mgm. per kilogram. As in making the cava pocket the abdominal aorta is tied near the bifurcation, a portion of the animal is excluded from the circulation. This has not been taken into account in calculating the dosage, nor do we know that any allowance should be made for it, as the structures mainly acted upon by nicotine and the other drugs studied are not affected by this ligation.

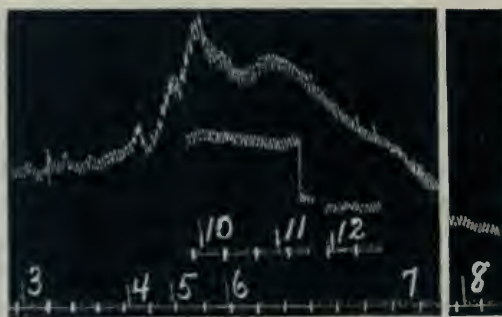


FIG. 19A. BLOOD PRESSURE TRACING. CAT 311

3, End of collection of second adrenal specimen; 4 to 5 intravenous injection of 0.4 mgm. nicotine; 6, beginning of collection of third adrenal specimen; 7, beginning of collection of fourth specimen; 8, beginning of collection of fifth specimen; 10, beginning of collection of sixth specimen; 11, beginning of collection of seventh specimen; 12, end of collection of seventh specimen. Zero line corresponds with time trace and is moved up 18 mm. and the figure then reduced to two-thirds.

With these small doses just as with the larger doses, the depressant action was the predominant and the most easily demonstrated effect. Indeed, relatively to the excitation the depression was more manifest than with the larger doses. The condensed protocol of one of the experiments is given as an example.

Condensed protocol. Cat 311, male, weight, 3.89 kgm.

Anesthetized with ether. Obtained indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

- 10.27 a.m. First specimen, 2.9 grams in 30 seconds (5.8 grams per minute).
- 10.27½ a.m. Second specimen, 7.1 grams in 90 seconds (4.7 grams per minute). Blood pressure at end of collection of second adrenal specimen was 90 mm. of mercury (fig. 19a, observation 3).
- 10.34 a.m. Injected 0.4 mgm. nicotine intravenously (fig. 19a, observations 4 and 5).
- 10.34½ a.m. Third adrenal specimen, 6.7 grams in 60 seconds (6.7 grams per minute).
- 10.35½ a.m. Fourth adrenal specimen, 5.15 grams in 90 seconds (3.4 grams per minute).
- 10.37 a.m. Fifth adrenal specimen, 5.55 grams in 120 seconds (2.2 grams per minute).

Blood pressure at beginning of collection of third adrenal specimen was 136 mm. of mercury (fig. 19a, observation 6), at beginning of collection of fourth adrenal specimen 97 mm. (observation 7), at beginning of collection of fifth adrenal specimen 70 mm. (observation 8).

- 10.48½ a.m. Sixth adrenal specimen, 1.75 grams in 30 seconds (3.5 grams per minute).
- 10.49 a.m. Seventh adrenal specimen, 7.45 grams in 180 seconds (2.5 grams per minute).

Blood pressure at beginning of collection of sixth specimen was 84 mm. of mercury (fig. 19a, observation 10), at beginning of collection of seventh specimen 76 mm. (observation 11), at the end of collection of seventh specimen 54 mm. (observation 12). Obtained another specimen of venous blood. Combined weight of adrenals 0.668 gram.

The second specimen, (before nicotine) corresponded to an output of epinephrin of 0.0006 mgm. per minute for the cat, or 0.00015 mgm. per kilogram per minute. The third specimen (collected twenty seconds, or allowing for the dead space, ten to fifteen seconds after the end of the nicotine injection) corresponded to an output of 0.00065 mgm. per minute for the cat, or 0.00016 mgm. per kilogram per minute, practically the same as the original output. That some augmentation was present in the early part of the period of collection of this specimen was indicated clearly by the fact that the fourth specimen, taken immediately after the third, corresponded to an output of only

0.00025 mgm. per minute for the cat, or 0.00006 mgm. per kilogram per minute, less than half the rate for the third specimen. Since it is very improbable that the depressant action began precisely with the collection of the fourth specimen, some augmentation must have been present in the early part of the third specimen, to make up for the depression during the latter part.

The fifth specimen, taken immediately after the fourth (beginning three minutes after the nicotine injection) already showed some recovery in the epinephrin output as compared with the fourth specimen (to 0.00044 mgm. per minute for the cat, or 0.00011 mgm. per kilogram per minute). When the seventh specimen was obtained (fifteen minutes after the nicotine injection) the original output had been regained (0.0006 mgm. per minute for the cat, or 0.00015 mgm. per kilogram per minute), although the blood pressure was considerably lower than at the time of collection of the second specimen. It must be remembered that in an experiment of this kind, the complete restoration of conductivity in the vasomotor efferent path, does not necessarily carry with it restoration of the blood pressure to the original level. The animal has been losing some blood. It has been kept under an anesthetic after an operation, and the blood pressure under these conditions may be gradually falling. The removal of the nicotine block will not then of course restore the pressure completely, although the epinephrin output may come back to the initial amount. The more prompt recovery with the smaller doses is what might be expected.

EXPERIMENTS WITH HYPODERMIC INJECTION OF NICOTINE AND ASSAY OF ADRENAL BLOOD ON RABBIT SEGMENTS

Two experiments were made with hypodermic injection of nicotine, in order to see whether with a more gradual action of the poison, the preliminary period of increased output might not be prolonged relatively to the succeeding period of depression. This was not found to be the case. On the contrary, the depressant effect of nicotine upon the epinephrin output asserted itself even more obviously than with intravenous injection, as the outstanding action of the drug upon the secretion.

Condensed protocol. Cat 303, male, weight, 4.82 kgm.

Anesthetized with ether. Obtained indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

10.27 a.m. First specimen, 3.5 grams in 30 seconds (7 grams per minute).

10.27½ a.m. Second specimen, 9.05 grams in 90 seconds (6 grams per minute).

Blood pressure during collection of second specimen was 136 mm. mercury (fig. 20, observation 4).

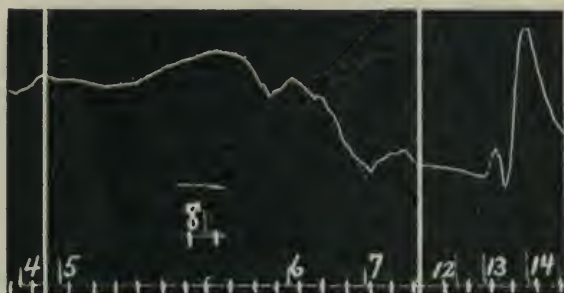


FIG. 20. BLOOD PRESSURE TRACING. CAT 303

4, End of collection of second adrenal specimen; 5, injected 5 mgm. nicotine subcutaneously; 6, beginning of collection of third adrenal specimen; 7, beginning of collection of fourth adrenal specimen; 8, beginning of collection of fifth adrenal specimen; 12, end of collection of seventh adrenal specimen; 13, injected 1 mgm. nicotine intravenously; 14, beginning of collection of eighth adrenal specimen. Zero line corresponds with time trace and is moved up 20 mm. and the figure then reduced to two-thirds.

10.34 a.m. Injected 5 mgm. nicotine subcutaneously (fig. 20, observation 5).

10.36 a.m. Third adrenal specimen, 2.55 grams in 30 seconds (5.1 grams per minute).

10.36½ a.m. Fourth adrenal specimen, 3.6 grams in 90 seconds (2.4 grams per minute).

10.38 a.m. Fifth adrenal specimen, 5.25 grams in 150 seconds (2.1 grams per minute).

Blood pressure at beginning of collection of third specimen was 120 mm. mercury (fig. 20, observation 6); at the beginning of collection of the fourth specimen 83 mm. mercury (fig. 20, observation 7); at the

beginning of collection of the fifth specimen 60 mm. mercury (observation 8).

10.51 a.m. Sixth adrenal specimen, 2.4 grams in 30 seconds (4.8 grams per minute).

10.51½ a.m. Seventh adrenal specimen, 8.3 grams in 120 seconds (4.2 grams per minute).

Blood pressure 88 mm. mercury (fig. 20, observation 12).

10.53¼ a.m. Injected 1 mgm. nicotine intravenously (fig. 20, observation 13).

10.54 a.m. Eighth adrenal specimen, 3.95 grams in 60 seconds (3.95 grams per minute).

10.55 a.m. Ninth adrenal specimen, 2.2 grams in 120 seconds (1.1 grams per minute).

At the beginning of the collection of the eighth specimen the blood pressure was up to 144 mm. mercury (fig. 20, observation 14); then it fell to 45 mm. mercury. Obtained another specimen of venous blood. Combined weight of adrenals 0.66 gram.

In the first of these experiments (cat 303), with a relatively large dose of nicotine (5 mgm.) the stimulation effect was missed altogether even in the adrenal blood specimen collected two minutes (about one and three-quarter minutes, allowing for the dead space) from the beginning of the nicotine injection. Further experiments showed, however, that a period of increased output would unquestionably have been detected in this cat had the first adrenal sample after nicotine been taken earlier. Figure 20 indicates the positions of the adrenal blood specimens on the blood pressure tracing.

The second specimen (taken before injection of the drug) was found to be stronger than 1:6,660,000, and weaker than 1:5,300,000 adrenalin (confirmed for each limit by 2 separate pairs of observations made at different times with the same intestine segment). Taking the second specimen at 1:6,000,000, we get 0.001 mgm. of epinephrin per minute for the cat, or 0.0002 mgm. per kilogram per minute.

The third specimen, collected two minutes after the hypodermic nicotine injection (one and three-quarter minutes, allowing for the dead space) was found to have a somewhat greater concentration of epinephrin than the second. It was distinctly

weaker than 1:4,000,000, stronger than 1:6,660,000, and was assayed at 1:5,200,000, equivalent to an output of 0.001 mgm. per minute for the animal, or 0.0002 mgm. per kilogram per minute, the same as for the second specimen.

Subsequent experience enables us to interpret these results. They do not mean that the relatively large dose of nicotine administered hypodermically produced no effect upon the epinephrin output, but that a brief period of increased output had intervened, somewhere between the injection and the beginning of collection of the third specimen. This increased output lapped over to some extent into this sample, maintaining approximately the same output as in the second specimen in the face of the rapidly developing depression. It is not known whether the depression had reached its maximum before collection of the seventh specimen (seventeen and one-half minutes after the nicotine injection) or was still developing. Probably the maximum depression occurred somewhere between the fourth and seventh specimens for the blood pressure had recovered considerably when the latter was collected. The seventh adrenal specimen had a much smaller concentration of epinephrin than the fourth, but the blood flow was nearly twice as great. The seventh was somewhat weaker than the second. It was weaker than 1:6,660,000, somewhat stronger than 1:8,000,000, and was taken at 1:7,200,000, corresponding to an output of 0.0006 mgm. per minute for the cat, or 0.00012 mgm. per kilogram per minute. At this time then the output had not yet reached its original value before nicotine.

At this point a dose of nicotine (1 mgm.) was injected intravenously in order to ascertain whether the transient preliminary stimulating effect could be demonstrated in this animal when an adrenal blood sample was collected immediately after the injection. The result was positive, as in all the experiments with intravenous injection. The eighth specimen was collected fifteen seconds, or allowing for the dead space, only a few seconds after the nicotine injection. The pocket had been clipped off before the collection of the sixth specimen, and the adrenal blood was continuously collected till the end of the ninth specimen.

The nicotine was injected while collection was going on, and collection of the eighth specimen was begun at the moment when the blood pressure began to rise after the nicotine injection. The fact that an excellent rise of pressure was produced by the nicotine, although no epinephrin was entering the circulation, or had entered it for three minutes, is of interest in connection with Gley's theory, already criticized in the introduction to this paper, of the mode of action of nicotine in increasing the blood pressure.

The eighth adrenal blood specimen was proved by the assay to be weaker than 1:1,300,000, stronger than 1:2,700,000, and not far from 1:2,000,000 epinephrin, equivalent to an output of 0.002 mgm. per minute for the cat, or 0.0004 mgm. per kilogram per minute, more than three times the output just before the intravenous injection and twice the output at the beginning of the experiment.

In the other experiment with hypodermic injection of the drug (cat 305) a smaller dose was employed and the adrenal blood was collected at a shorter interval after the injection.

Condensed protocol. Cat 305, male, weight, 3.41 kgm.

Anesthetized with ether. Obtained indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

10.53½ a.m. First specimen, 1.6 grams in 30 seconds (3.2 grams per minute).

10.54 a.m. Second specimen, 6.8 grams in 120 seconds (3.4 grams per minute).

Blood pressure during collection of the second specimen was 140 mm. mercury (fig. 21, observation 4).

11.00 a.m. Injected 2 mgm. nicotine subcutaneously (fig. 21, observation 5).

11.01 a.m. Third adrenal specimen, 4.25 grams in 60 seconds (4.25 grams per minute).

11.02 a.m. Fourth adrenal specimen, 4 grams in 90 seconds (2.7 grams per minute).

11.03½ a.m. Fifth adrenal specimen, 4.9 grams in 150 seconds (1.96 grams per minute).

Blood pressure at the beginning of the collection of the third specimen was 172 mm. mercury (fig. 21, observation 6); at the beginning of the collection of the fourth specimen, 154 mm. mercury (observation 7); and at the beginning of the collection of the fifth specimen 108 mm. mercury (observation 8).

11.18 a.m. Sixth adrenal specimen.

11.18½ a.m. Seventh adrenal specimen, 3.6 grams in 180 seconds (1.2 grams per minute).

Blood pressure at the end of collection of the seventh specimen was 76 mm. mercury (fig. 21, observation 12).

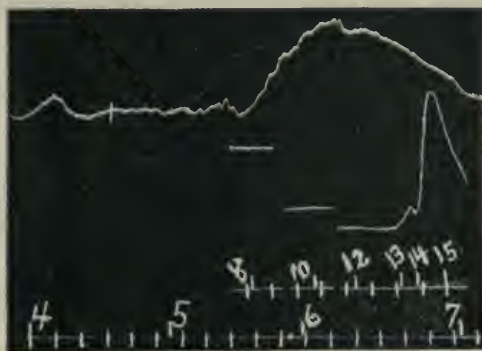


FIG. 21. BLOOD PRESSURE TRACING. CAT 305

4, End of collection of second adrenal specimen; 5, subcutaneous injection of 2 mgm. nicotine; 6, beginning of collection of third adrenal specimen; 7, beginning of collection of fourth adrenal specimen; 8, beginning of collection of fifth adrenal specimen; 10, beginning of collection of sixth adrenal specimen; 12, end of collection of seventh adrenal specimen; 13 to 14, intravenous injection of 1 mgm. nicotine; 15, beginning of collection of eighth adrenal specimen. Zero line corresponds with time trace and is moved up 26 mm. and the figure then reduced to two-thirds.

11.22 a.m. Injected 1 mgm. nicotine intravenously (fig. 21, observations 13 to 14).

11.22¼ a.m. Eighth adrenal specimen, 1.5 grams in 60 seconds (1.5 grams per minute).

The collection of the eighth specimen was begun at the height of the nicotine rise when the blood pressure had reached a maximum of 130 mm. mercury (fig. 21, observation 15). Allowing for the dead space, the

first drops of the eighth specimen must have left the adrenals about the time the rise was beginning.

Obtained another specimen of venous blood. Combined weight of adrenals 0.312 gram.

The points on the blood pressure curve at which the adrenal blood specimens were procured are indicated in figure 21.

The second adrenal specimen, collected before hypodermic injection of nicotine, was shown by the assay to be much weaker than 1:4,300,000 adrenalin, weaker than 1:5,700,000, stronger than 1:7,000,000. It was confirmed by other observations that the concentration of the specimen was between 1:7,000,000, and 1:5,700,000, and nearer the latter. It was taken at 1:6,000,000, corresponding to an output of 0.00057 mgm. per minute for the cat, or 0.00017 mgm. per kilogram per minute.

The third specimen was procured about a minute after the injection (about twenty seconds after the beginning of the rise of pressure, or only five to ten seconds after the beginning of the rise when the filling of the dead space is corrected for). It was found to be stronger than 1:4,300,000 adrenalin, and decidedly weaker than 1:3,000,000. It was finally assayed at 1:3,500,000 equivalent to an output of 0.0012 mgm. per minute for the cat, or 0.00035 mgm. per kilogram per minute, about double the output before the nicotine injection.

The fourth specimen, collected immediately after the third, beginning about two minutes after the administration of nicotine and little more than a minute after the commencement of the rise of pressure, gave practically the same reaction with the intestine segment as the third specimen (confirmed by two pairs of observations). It was proved to be stronger than 1:4,300,000, weaker than 1:3,000,000 adrenalin, and approximately equivalent to 1:3,500,000. As the blood flow for the third specimen was 50 per cent greater than for the fourth, the output was already diminished at this time, amounting to 0.0008 mgm. per minute for the cat, or 0.00023 mgm. per kilogram per minute. As the next adrenal specimen, the fifth, taken immediately after the fourth, caused no inhibition of the intestine segment, although the blood flow was smaller, it is practically certain that a marked depres-

sion of the epinephrin liberation was already in evidence during the latter part of the period of collection of the fourth specimen, the epinephrin content of this specimen being largely accounted for by the overlapping of the period of stimulation into the first part of the fourth specimen. It is not known, of course, but from other experiments it is probable that during the first half minute of the period of collection of the third specimen the output was materially greater than during the second half minute. What is beyond doubt is that about two and one-half minutes after the absorption of the nicotine had reached the point when an effect on the blood pressure was beginning to manifest itself, the depressant action was already so strong that an intestine segment which could easily have detected a concentration of 1:10,000,000 epinephrin (in blood made up to this concentration and then diluted with three volumes of Ringer's solution) and indeed considerably less than this, gave no inhibition whatever with the fifth specimen. The output at this time accordingly could not have been as much as 0.0002 mgm. per minute for the cat, or 0.00006 mgm. per kilogram per minute, i.e., not one-third of the output before nicotine. The segment was not very sensitive, so that it is impossible to know how much below this the output had sunk.

Fourteen and a half minutes later, i.e., eighteen and one-half minutes after the nicotine injection, the depressant action was still so strong that the seventh adrenal blood specimen also caused no inhibition of the intestine segment (fig. 22, observation 69), although the blood flow was slower than for the fifth specimen. Indifferent blood made up with adrenalin to 1:7,000,000, and then diluted to the same extent as the seventh specimen (with three volumes of Ringer's solution) gave a definite inhibition, although with 1:14,000,000 there was no reaction at this time (fig. 22, observations 71 and 73). It was shown that the seventh specimen could not have contained 1:10,000,000 epinephrin. The output at this time accordingly must have been less than 0.00012 mgm. per minute for the cat, or 0.000035 mgm. per kilogram per minute, i.e., less than one-fifth of the output before nicotine. Subcutaneous injection,

then, instead of accentuating the preliminary excitation of nicotine on the epinephrin output at the expense of the depressant effect, brings out the latter more clearly than with intravenous injection, as the predominant action of the drug. It is not known how much longer the depressant effect would have lasted. That it was not over at the end of collection of the seventh specimen is indicated by the fact that the blood pressure had not yet begun to recover.

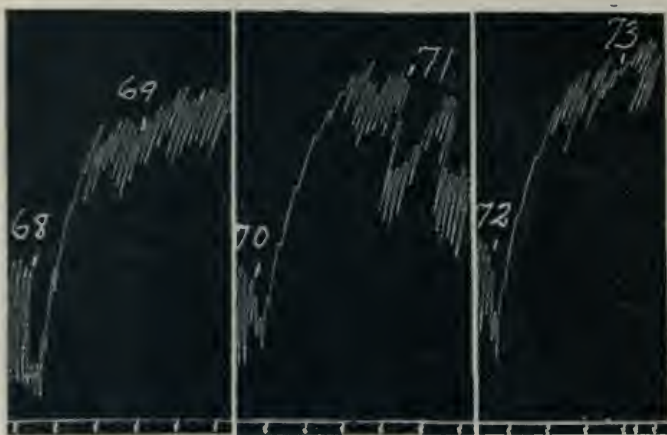


FIG. 22. INTESTINE TRACINGS. BLOODS FROM CAT 305

At 68, 70 and 72 Ringer was replaced by venous blood obtained after injection of nicotine; and this at 69 by the seventh adrenal specimen (collected eighteen minutes after subcutaneous injection of nicotine); at 71, by venous blood to which was added adrenalin to make a concentration of 1: 7,000,000; at 73 by venous blood to which was added adrenalin to make a concentration of 1: 14,000,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to two-thirds.)

Immediately after collection of the seventh specimen, an intravenous injection of nicotine was made and the eighth adrenal specimen collected within fifteen seconds of the beginning of the injection, or allowing for the dead space, almost simultaneously with the completion of the injection, and just as the blood pressure was beginning to rise. The eighth specimen was found to be much stronger than 1: 1,400,000, somewhat stronger than

1:700,000, much weaker than 1:430,000 adrenalin (fig. 23). It was taken at 1:600,000, corresponding to an output of 0.0025 mgm. epinephrin per minute for the cat, or 0.0007 mgm. per kilogram per minute. This is four times the initial output before the subcutaneous injection of nicotine. In spite of the depression of the secretory path just before the intravenous injection, the depression was at once changed into excitation, just as in the case of the vasomotor peripheral neurones.

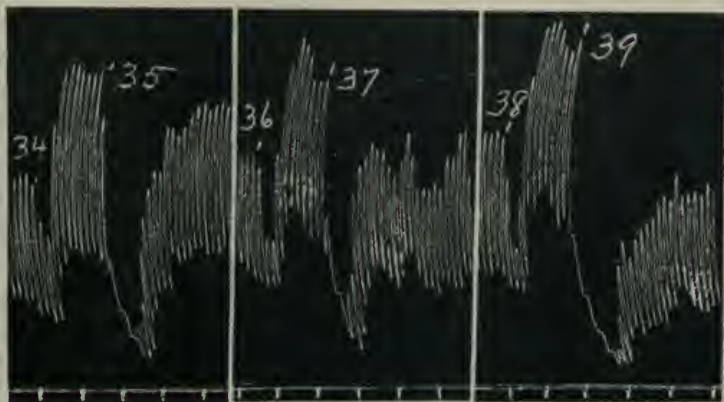


FIG. 23. INTESTINE TRACINGS. BLOODS FROM CAT 305

At 34, 36 and 38 Ringer was replaced by venous blood obtained after injection of nicotine; and this at 35 by venous blood to which was added adrenalin to make a concentration of 1:700,000; at 37 by the eighth adrenal specimen (collected immediately after intravenous injection of nicotine); at 39 by venous blood to which was added adrenalin to make a concentration of 1:430,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to two-thirds.)

The percentage of serum in the blood was 54, as determined by the electrical method; (by the hematocrite, 43 with ten minutes, 48.5 with twenty minutes, and 51.5 with thirty-two and one-half minutes rotation). The concentration of epinephrin in the serum of the eighth specimen was, therefore, about 1:320,000.

To sum up: the experiments made by the direct method of assaying the adrenal blood, collected at a known rate, on rabbit segments, show that the action of nicotine, whether administered

intravenously or hypodermically, upon the epinephrin output of the adrenals is essentially a depressant or paralysing action, which comes on early, lasts a relatively long time and only gradually, according to the dose and to other conditions, disappears. The liberation of epinephrin during the period of maximum depression is reduced to a small fraction of the original output, or within the limits of sensitiveness of the test objects, the discharge may cease entirely just as it does when all the secretory fibres are cut. The depressant action is preceded by a transient stimulation during which the rate of output of epinephrin is increased. The duration of the period of excitation probably varies to some extent with the dose, the method of administration and other circumstances, but in general it is measured by half or quarter minutes rather than by minutes, whereas the stage of depression, with the doses employed, may last for many minutes. It was rare to find evidence of the stage of excitation lasting as long as one minute. Because of overlapping of the blood specimens it would require very numerous experiments to fix precise limits for so brief an effect. But in the majority of the experiments the duration of the increased epinephrin output was from half a minute to a minute.

Although it would be difficult to obtain and to assay a sufficient number of adrenal blood samples to make a good curve representing the changes in the rate of epinephrin output throughout an experiment, there is little doubt that such a curve would be roughly parallel to the curve of blood pressure, indicating that the nicotine action on the sympathetic ganglion cells of the efferent vasomotor path is similar to the nicotine action on the sympathetic ganglion cells, or whatever structures may represent them, if there are no sympathetic ganglion cells (6), on the path of the fibres which govern the liberation of epinephrin.

It is obvious from the above results that writers (7), who state that a marked augmentation of the epinephrin discharge in cats is present from three or four to ten or twelve minutes after the intravenous injection of nicotine in doses not very different from the highest doses used by us and that the augmentation may indeed be more pronounced after the longer interval, while making no mention of any depressant effect, must

have employed a faulty method. The period of augmentation must have been over even before they collected their earlier blood specimens after nicotine. The arterial blood pressure and the blood flow in the cava being much reduced, however, they might easily obtain samples of blood after nicotine with a greater epinephrin concentration than the specimen taken before the nicotine injection. The later specimens would be quite likely to have a greater concentration than the earlier specimens after nicotine, because the depressing effect of the drug on the output would probably have been recovered from to some extent when the later specimens were collected. But as already pointed out, the slowing of the circulation would necessarily augment the concentration if no increase occurred in the rate of epinephrin output, or even if a decrease occurred which was proportionally less than the decrease in the rate of blood flow. In the absence of information as to changes in the rate of the blood flow, which cannot be obtained by the catheter method, no conclusions can be drawn as to changes in the rate of output of epinephrin from changes in its concentration, even in the pure adrenal vein blood.

Two or three years ago, being curious to know at first hand what effects nicotine might produce upon the concentration of epinephrin in the cava blood collected in the neighborhood of the adrenals, although aware that information could not be obtained in this way as to changes in the rate of output of epinephrin, we performed several experiments by the catheter method. These are of interest in the present connection, and a condensed protocol of one of them (cat 10) with a small sample of the tracings from the intestine segment (fig. 24) is reproduced. In this experiment, and in some of the others we opened the abdomen, so as to be able to clip the adrenal veins at will, or to leave them free. The results did not differ essentially whether the abdomen was opened or not.

Condensed protocol. Cat 10, female, weight, 2.95 kgm.

Anesthetized with ether. Exposed both femoral veins; opened abdomen and isolated both adrenal veins.

Inserted catheter 40 mm. into right femoral vein and obtained first specimen.

Inserted catheter up to level of adrenals and obtained second specimen. Injected into femoral vein 5 mgm. nicotine, and 3 minutes later inserted catheter up to level of adrenals and obtained third specimen. Artificial respiration begun during the collection of third specimen. Now

clipped off both adrenal veins, and collected through a catheter the fourth specimen (9 minutes after collection of third specimen).

Five minutes after collecting the fourth specimen (i.e., 17 minutes after the nicotine injection) obtained from catheter at the adrenal level the fifth specimen. The blood pressure at this stage was very low. A cava pocket was made and a small sample of adrenal vein blood collected.

A sample of arterial blood was finally obtained. .

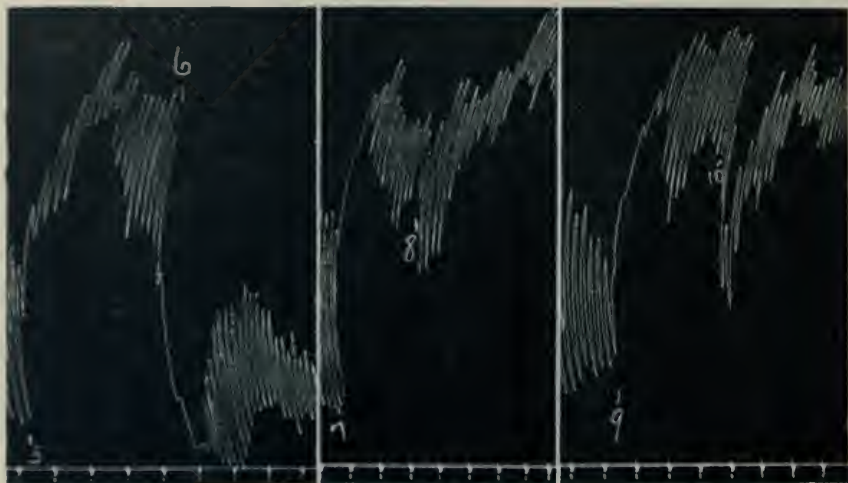


FIG. 24. INTESTINE TRACINGS. BLOODS FROM CAT 10

At 5 Ringer was replaced by catheter blood obtained from the lower cava, and this at 6 by adrenal vein blood; at 7 and 9 Ringer was replaced by catheter blood drawn from the cava at adrenal level after nicotine injection but after clipping off both adrenal veins, and this at 8 by catheter blood obtained from the level of the adrenals (with adrenal veins open) three minutes after injection of nicotine; at 10 by catheter blood obtained from the same level (with adrenal veins open) seventeen minutes after the nicotine injection.) (Reduced to one-half.)

The catheter was rinsed with Ringer's fluid and freshly oiled between collection of the specimens. The collections were made by aspirating slowly with the aid of a syringe.

Cava blood from the adrenal level before nicotine, displacing the lower cava blood, gave slight inhibition of the intestine segment, while cava blood from the adrenal level 3 minutes after the injection caused no effect. To eliminate any action of the nicotine in the blood on the segment, observations were then made in which cava blood from the

adrenal level, obtained with the adrenal veins clipped, was displaced by cava blood from the same level with the adrenal veins open. The specimen taken 3 minutes after injection of nicotine, gave no inhibition of the segment, whereas the specimen obtained 17 minutes after the nicotine injection caused a fair inhibition (fig. 24, observations 8 and 10).

The same result was obtained when indifferent blood drawn with the catheter from the lower part of the cava, was displaced by the 3 minute and the 17 minute specimens, the former gave no reaction, the latter a fair effect. When the 3 minute specimen was displaced by the 17 minute specimen, a good inhibition was produced, confirming the conclusion that the concentration of epinephrin was greater in the latter. No attempt was made to assay the concentration in the 17 minute specimen, as in the absence of data on the rate of blood flow no conclusion could have been arrived at in regard to the effect of nicotine upon the epinephrin output, but it was shown that it was much less than 1:7,000,000, and, of course, very much less than that of pure adrenal vein blood (fig. 24, observation 6). Tests with the rabbit uterus confirmed the intestine observations as to the relative concentration of epinephrin in the various specimens.

EXPERIMENTS WITH AUTO-ASSAY BY BLOOD PRESSURE REACTIONS

Although the results of the experiments in which adrenal vein blood was collected and assayed on rabbit segments were unequivocal, it seemed desirable to confirm them by other methods. The next experiment (cat 299) is an example of such confirmatory observations made by a method of auto-assay (collection of adrenal blood in a cava pocket for a definite time before and after administration of nicotine, with subsequent release of the blood into the circulation). It was easier to obtain satisfactory curves than with strychnine for reasons already mentioned in the paper on that drug. Some samples of the blood pressure tracings are reproduced in figures 25 to 28. The animal, a male cat weighing 4.18 kgm. (the adrenals weighed 0.761 gram) was anesthetized with urethane. A "long" cava pocket was formed, the abdominal aorta, but not the intestinal arteries, being tied. A blood pressure tracing was taken from a carotid. Numerous pocket experiments were made to determine the output of epinephrin and the effect of nicotine upon it. At 5 (fig. 25) is shown

the effect of the adrenal blood collected for two minutes before nicotine, at 9, the effect of the adrenal blood collected for the same time after intravenous injection of 1 mgm. of nicotine. The nicotine was injected immediately after the pocket was closed off. The amount of epinephrin discharged into the circulation at 9 was obviously much greater than at 5. At 11, a two minute pocket was opened and caused a much smaller effect on the blood pressure than was caused by the opening of a pocket of the same duration before the nicotine injection. At this time,

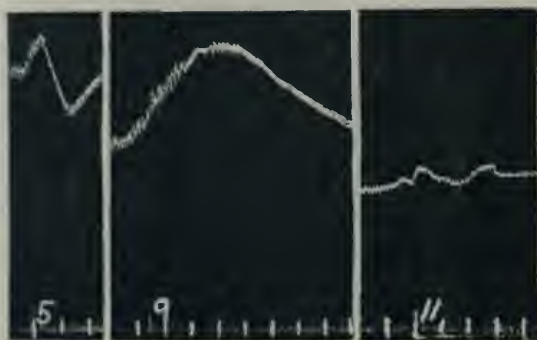


FIG. 25. BLOOD PRESSURE TRACING. CAT 299.

At 5, a two minute pocket (before injecting nicotine) was released; at 9, a two minute pocket, during period of closure of which was injected 1 mgm. nicotine, was released; at 11, a two minute pocket (seven minutes after the nicotine injection) was released. Zero line corresponds with time trace and is moved up 30 mm. and the figure then reduced to two-thirds.

therefore, five to seven minutes after the nicotine injection, the stage of depression was still pronounced.

In figure 26 is shown the effect of releasing a pocket which had been closed for one minute. Immediately after the closure of the pocket, 1 mgm. of nicotine was injected into the jugular vein. This was fourteen minutes after the first injection of nicotine. The rise of pressure, caused by the nicotine, was already over before the pocket was opened, so that the adrenal blood collected in it would contain the increased amount of epinephrin liberated during the transient period of excitation. At 16 the

pocket was opened. The effect on the blood pressure was less than that produced by 0.5 cc. of a 1:33,000 solution of adrenalin injected between 21 and 22, greater than that produced by 0.5 cc. of a 1:66,000 solution of adrenalin injected between 19 and 20, much greater than the effect produced by 0.5 cc. of a 1:130,000 solution of adrenalin injected between 17 and 18. Taking the effect as equivalent to that of 0.5 cc. of a 1:50,000 solution of adrenalin, we get 0.01 mgm. per minute for the cat, or 0.0025 mgm. per kilogram per minute, as the output of epinephrin during

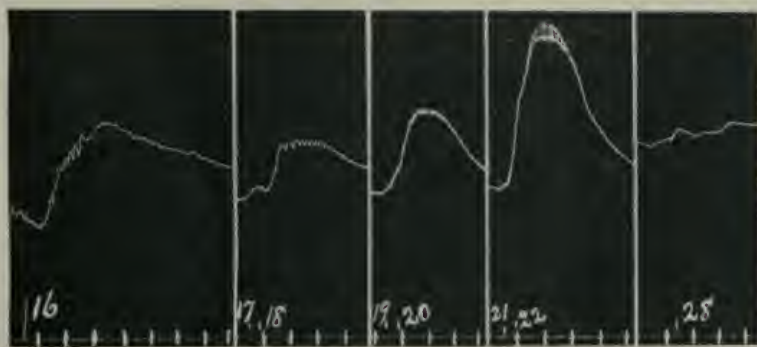


FIG. 26. BLOOD PRESSURE TRACING. CAT 299

At 16, a one minute pocket, during period of closure of which 1 mgm. nicotine was injected, was released; 17 to 18, injected 0.5 cc. of 1:130,000 adrenalin; 19 to 20 injected 0.5 cc. of 1:66,000 adrenalin; 21 to 22, injected 0.5 cc. of 1:33,000 adrenalin; at 23 a one minute pocket was released. Zero line corresponds with time trace and is moved up 22 mm. and the figure then reduced to two-thirds.

the excitation caused by nicotine. This is ten times the normal average in urethanized cats. Probably the increase was still greater in the first half minute after the nicotine injection. It was found, as a matter of fact, that when nicotine was injected intravenously immediately after closing off a pocket, the effect produced on the blood pressure by release of the pocket after one minute, was almost as great as after two minutes, showing that nearly the whole of the epinephrin was liberated in the first minute.

A one-minute pocket, sixteen and one-half minutes after the second nicotine injection (fig. 26, observation 28), caused a very small effect in comparison with the effect at 16, the stage of depression not being entirely recovered from.

A third injection of nicotine was made twenty-three minutes after the second and in the same manner. Although no assay was made of the epinephrin output following this injection the results were qualitatively precisely the same as before, the brief stage of excitation followed by the prolonged depression being equally well marked. The vagi were cut, shortly before the third nicotine injection.

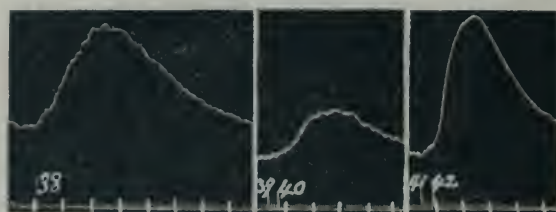


FIG. 27. BLOOD PRESSURE TRACING. CAT 299

At 38 a one minute pocket (during the closure of which 1 mgm. of nicotine was injected) was released; 39 to 40, injected 0.5 cc. of 1:66,000 adrenalin; 41 to 42 injected 0.5 cc. 1:33,000 adrenalin. Zero line corresponds with time trace and is moved up 30 mm. and the figure then reduced to two-thirds.

A fourth injection of 1 mgm. of nicotine was made nine minutes after the third, immediately after clipping off a pocket, which was released at 38 (fig. 27) after having been closed for one minute. The rise of pressure produced was much greater than that caused by 0.5 cc. of 1:66,000 adrenalin (fig. 27, observations 39 and 40) slightly less, as regards the maximum rise, but of greater duration than the rise caused by 0.5 cc. of 1:33,000 adrenalin. If the epinephrin liberated in one minute under the influence of nicotine be taken as equivalent to 0.5 cc. of a 1:40,000 solution of adrenalin, we get an output of 0.0125 mgm. per minute for the cat, or 0.003 mgm. per kilogram per minute, i.e., twelve times the average normal output.

Half an hour after the fourth injection of nicotine, when the blood pressure had fallen to about 50 mm. of mercury, and the curve was maintaining a uniform level, favorable for the detection of small epinephrin effects, a fifth injection of 1 mgm. was made immediately after the closure of the cava pocket. After being closed for two minutes the pocket was opened at 63 (fig. 28). The rise of pressure was much less than that produced by the release of a one minute pocket earlier in the experiment. But it has already been pointed out that this proves nothing at all as to a decrease in the amount of epinephrin present unless it be known that the sensitiveness of the test object to

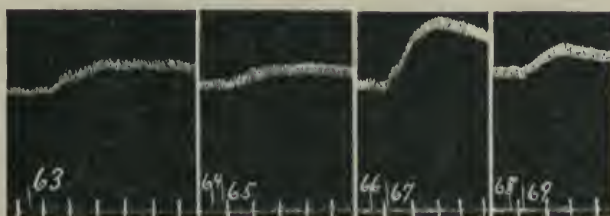


FIG. 28. BLOOD PRESSURE TRACING. CAT 299

At 63 a two minute pocket (during closure of which 1 mgm. of nicotine was injected) was released; 64 to 65, injected 0.5 cc. of 1:66,000 adrenalin; 66 to 67, injected 0.5 cc. of 1:33,000 adrenalin; 68 to 69 injected 0.5 cc. of 1:47,000 adrenalin. Zero line corresponds with time trace. (Reduced to two-thirds).

epinephrin has not decreased. In this case the sensitiveness had decreased decidedly. And the assay showed that a considerable transient augmentation of the epinephrin output had in reality been caused by the nicotine. For the effect was markedly greater than that produced by 0.5 cc. of 1:66,000 adrenalin, and not much different from that caused by 0.5 cc. of 1:47,000 adrenalin (fig. 28), corresponding to an output of 0.01 mgm. for the cat for the two minutes. This is practically the same as for one minute in the previous observations, and if it be remembered that the nicotine excitation effect almost certainly did not endure beyond one minute and probably not so long, it will be seen that even at this stage in the experiment and after so many

doses of nicotine, the transient augmentation of epinephrin output was still little, if at all, inferior to that obtained earlier. The long-lasting stage of depression followed regularly as before. During this stage several injections of strychnine (in all 2.5 mgm.) caused no demonstrable increase in the epinephrin output. Although no great weight can be attached to such a negative result obtained near the close of the experiment, it is altogether in harmony with our conception that strychnine in augmenting the epinephrin output acts upon the central mechanism. When the conductivity of the efferent path is depressed by nicotine, the central stimulation cannot make itself felt.

The results obtained by the above method of auto-assay, entirely corroborated the experiments in which the adrenal blood was assayed on rabbit segments, as to the course, duration and magnitude of the depressant and excitatory actions of nicotine upon the epinephrin output.

EXPERIMENTS ON (DENERVATED) EYE REACTIONS

To obtain corroboration by yet another method, an experiment was made to determine whether any difference existed between the eye reactions, after excision of the superior cervical ganglion, in an otherwise normal cat and in a cat whose epinephrin output had been interfered with by excision of one adrenal and denervation of the other.

Condensed protocol. Cat 300, male, weight 2.7 kgm.

Left superior cervical ganglion excised 44 days previously.

- 10.00 a.m. Left pupil contracted and nictitating membrane forward.
- 10.09 a.m. Injected subcutaneously 0.25 mgm. nicotine. The only symptom elicited was slight salivation.
- 10.48 a.m. Injected subcutaneously 2.5 mgm. nicotine. In 2 to 3 minutes, micturition, defaecation and vomiting occurred; the left pupil became maximally dilated, right dilating moderately.
- 11.00 a.m. Vomited; the left pupil dilated to maximal, but returned to previous state shortly after the paroxysm of vomiting.

- 11.02 a.m. Cat lying in cage quietly; the left pupil slightly wider than the right.
- 11.25 a.m. The pupils are equal; on exciting the cat, the left becomes wider than right, both dilating somewhat, but equality is soon again established.
- 12.00 m. Pupils equal; excitation causes the same phenomena noted at 11.25.

Condensed protocol. Cat 304, female, weight, 2.15 kgm.

Right adrenal excised; left adrenal denervated; left superior cervical ganglion excised; 46 days before the experiment. Condition excellent.

- 10.00 a.m. Left pupil contracted and nictitating membrane forward.
- 10.08 a.m. Injected subcutaneously 2.5 mgm. nicotine.
- 10.16 a.m. Vomited; during the vomiting paroxysm, both pupils dilated, but the left remained smaller than the right.
- 10.20 a.m. Vomited; pupils as in 10.16 observation.
- 10.25 a.m. Lachrymation, twitching of ears, salivation and erection of hair on tail.
- 11.40 a.m. Injected subcutaneously 2.5 mgm. nicotine.
- 11.42 a.m. Violent vomiting paroxysm; both pupils dilated, the left becoming slightly wider than the right and the left nictitating membrane retracting.
- 11.44 a.m. Left pupil still slightly wider than the right and left nictitating membrane still retracted.
- 11.45 a.m. Vomited; same phenomena as at 11.42.
- 12.30 p.m. Anesthetized with ether; obtained a specimen of jugular blood, then made cava pocket and collected adrenal blood specimens.
- 1.05 p.m. 1st specimen, 1.2 grams in 30 seconds (2.4 grams per minute)
2nd specimen, 6.05 grams in 240 seconds (1.5 grams per minute). 3rd specimen, 3.6 grams in 240 seconds (0.9 gram per minute).

Obtained a specimen of arterial blood.

It was recognized, as already remarked, that such comparisons yield information far less precise and far more difficult of interpretation than the other methods as to the influence of given conditions upon the epinephrin output. Nevertheless, as illustrated in the condensed protocols, a much greater and more durable dilatation of the pupil on the side from which the superior cervical ganglion had been removed, as compared with the normal pupil, was associated with the nicotine action in the nor-

mal cat (cat 300) than in the other (cat 304). Whether this was due to increased epinephrin liberation during the transient stage of excitation, renewed possibly more than once in the non-anesthetized animal as absorption of the poison proceeded, cannot be definitely known, although we have evidence that a maximal dilatation of the pupil of the denervated eye, once established by a large dose of epinephrin, is far more durable than a smaller dilatation, or than a maximal dilatation due to a smaller dose. Naturally under such complicated conditions the depressant action of the drug upon the epinephrin secretion can be studied, if at all, with great difficulty. For the pupil dilatation due to a single dose of adrenalin more than sufficient to produce a maximal effect, does not soon disappear even when all further access of epinephrin is prevented, although by quantitative comparisons under proper conditions it can be shown that the continuous normal discharge of epinephrin after the maximal dilatation has been produced delays measurably the return of the pupil to its original size. Paralysis of the ciliary ganglion, as Langley (5) has pointed out in discussing the experiments of Dale and Laidlaw (1) already referred to, may complicate such observations upon the effect of nicotine on the eye reactions. The difference between the normal cat and the cat whose epinephrin liberation had been interfered with in our observations could not, however, be accounted for in this way, since there is no known reason why the ciliary ganglion should have been affected differently in the two animals.

The epinephrin assay of the adrenal blood specimens collected in cat 304, after the observations on the eye had been completed, showed that both the second and the third specimens were much weaker than 1:6,700,000 adrenalin. The second specimen was weaker than 1:26,500,000, somewhat stronger than 1:40,000,000, about the same as 1:37,000,000, corresponding to an output of 0.00004 mgm. per minute for the cat, or 0.000018 mgm. per kilogram per minute. The third specimen was weaker than 1:20,000,000, much stronger than 1:66,000,000, about the same as 1:26,500,000, corresponding to an output of 0.000034 mgm. per minute for the cat, or 0.000015 mgm. per kilogram per minute, not more than one-sixteenth of the normal average output.

EFFECT OF NICOTINE ON THE EPINEPHRIN STORE

One experiment was made to determine whether nicotine had any detectable influence upon the amount of the epinephrin store of the adrenals. As the superior cervical ganglion had been previously excised on one side, the opportunity was taken to study the eye reactions in this cat also (cat 301) and for this reason the condensed protocol is given.

Condensed protocol. Cat 301; male; weight, 1.65 kgm.

Left adrenal denervated and left superior cervical ganglion excised nineteen days before the experiment.

- 11.00 a.m. Left pupil contracted and nictitating forward.
- 11.10 a.m. Injected subcutaneously 2 mgm. nicotine; in about two minutes the cat vomited.
- 11.18 a.m. Vomited; left pupil only slightly wider than the right.
- 11.27 a.m. Injected subcutaneously 2 mgm. nicotine.
- 11.30 a.m. Vomited; left pupil became maximally dilated, right moderately dilated.
- 11.33 a.m. Left pupil maximal.
- 11.40 a.m. Right pupil dilated to half the maximal; left pupil maximal; both nictitating membranes are partly forward.
- 12.10 p.m. Pupils equal (dilated to about one-fourth maximal), nictitating membranes forward.
- 1.18 p.m. Injected 2 mgm. nicotine.
- 1.22 p.m. Vomited; left pupil became much wider than right; after vomiting paroxysm the left came down to nearly the width of the right, but remained somewhat wider; the nictitating membrane remained forward. This dose did not cause as great an effect on the pupil as the previous doses.
- 2.35 p.m. Pupils equal. Injected subcutaneously 2.5 mgm. nicotine.
- 2.40 p.m. Vomiting paroxysm accompanied by transient dilatation of both pupils, the left becoming slightly wider than the right, but returning to equality very soon.
- 4.00 p.m. Injected 5 mgm. nicotine; same phenomena as at 1.22; twitching of ears present for the past hour.
- 4.45 p.m. Killed suddenly and removed adrenals.

Left adrenal weighed 0.15 gm. and contained 0.21 mgm. epinephrin; right adrenal weighed 0.165 grams and contained 0.21 mgm. epinephrin.

No change whatever was demonstrated in the epinephrin store, although the very large amount of 13.5 mgm. of nicotine was injected in 5 doses and the cat was under its influence for five and one-half hours.

SUMMARY

1. The predominant and by far the most durable action of nicotine, whether administered intravenously or hypodermically, upon the epinephrin output is a depressant or paralyzing action. The maximum diminution of the epinephrin output is rather rapidly reached and then there is a more gradual recovery, which when the dose is not too large, proceeds till the original output is approximately attained. At the time of maximum depression no epinephrin at all may be detected in the adrenal vein blood by the test objects chiefly employed (rabbit intestine and uterus segments).

2. The depressant action is preceded by a transient stage of excitation, lasting as a rule in these experiments not longer than from half a minute or less to a minute. In this stage the rate of epinephrin output is markedly increased (from two or three to ten or fifteen times the original output or even more, under our experimental conditions).

3. The latent period of the transient excitation, with intravenous injection of the drug, is very short. In some of the experiments there was evidence that it could not have exceeded a few seconds.

4. The brief stage of excitation passes rather abruptly into the much more durable stage of depression. The maximum increase in the rate of epinephrin output is followed at a relatively short interval by the maximum depression of the rate, after which begins the gradual recovery.

5. The changes in the rate of epinephrin output are roughly parallel to the changes in the blood pressure caused by nicotine, indicating that when the sympathetic ganglion cells on the efferent vasomotor path are being stimulated or depressed, a corresponding stimulation or depression is being exerted on the efferent adrenal secretory path.

6. It may be pointed out that the nicotine effect on the epinephrin output is, speaking generally, the converse of the strychnine effect (see paper 1 of this series). The predominant action of strychnine is a marked and long lasting augmentation of the epinephrin output. There are indications that the strychnine stimulation of the output may be preceded by a brief depression. The nicotine action develops more suddenly than the strychnine action, as might be expected from the fact that the point of attack of nicotine is the efferent path, of strychnine the central mechanism.

7. The transient augmentation of the epinephrin output by nicotine may be associated with an increase in the concentration of epinephrin in the adrenal vein blood much beyond the maximum seen with the slowest blood flows in animals simply anesthetized (with ether, morphine or urethane). The strychnine augmentation of the output has not been observed to be associated with any increase in the normal maximum concentration (something like 1:500,000 in the serum of adrenal blood, assayed with rabbit segments).

8. Confirmatory evidence of the conclusions deduced from assays of the adrenal blood on rabbit intestine (and uterus) segments has been obtained by a method of auto-assay (collection of adrenal blood for a given time in a cava pocket and study of the blood pressure reactions elicited when the blood is released from the pocket into the circulation), and by other methods.

9. In the one experiment performed the epinephrin store of the adrenals was not found to be altered by nicotine.

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ON THE PRESENCE OF HISTAMINE (β -IMINAZOLYL-ETHYLAMINE) IN THE HYPOPHYSIS CEREBRI AND OTHER TISSUES OF THE BODY AND ITS OCCURRENCE AMONG THE HYDROLYTIC DECOMPOSITION PRODUCTS OF PROTEINS*

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During the past four years, investigations on the chemical and pharmacological properties of the gastric and intestinal mucosa (1) have been in progress in our laboratory. In previous papers some of the results obtained were described. The so-called "peristaltic hormones" or "motilines," which European investigators have shown to be present in extracts of the intestinal mucosa and whose chemical nature has been entirely unknown up to the present, were among the principles that first engaged our attention. In one of the papers (2) mentioned above, we stated,

It is not our purpose to consider here the literature pertaining to the "peristaltic hormones" that are known to occur in almost all, if not all, organs of the body. It is worthy of note, however, that an extract of the gastric or intestinal mucosa can be prepared, as we have already stated, which has a pressor action for the circulation and a marked oxytoxic power in a concentration of 1:1,000,000. This powerful action points strongly to the conclusion that here also, as in the case of pituitary extracts, we are dealing with a motiline, which, in a state of chemical purity, would be fully as active as β -iminazoly-ethylamine. And this again leads us to the supposition that the oxytoxic principle (or motiline) of the hypophysis is not a hormone or substance specific to this organ, but is a rather widely distributed substance, everywhere the same, which may have its origin in the various tissues, in the gastric or intestinal mucosa, or which may be absorbed as such from among the products of digestion.

* Read before the American Society for Pharmacology and Therapeutics at Baltimore, April 25th, 1919.

The ideas here outlined were further elaborated in a second paper which was primarily concerned with the occurrence of proteoses in animal tissues, but which also gave directions for the removal of such highly active constituents as secretin and "motiline," from albumoses. The well known Witte's peptone, so often used in the past to induce experimentally a state of collapse known as "peptone shock," has made us familiar with the fact that proteoses are either toxic in themselves, as has often been maintained, or else retain, firmly attached to themselves, smaller and highly toxic molecules which exist quite independently in the medium from which the "peptones" were obtained. The latter view is the one held by us and a study of our earlier papers will show that in all instances of the kind here indicated it was possible to purify a given albumose so that it no longer exhibited pharmacological activity, pressor, oxytoxic, or secretory.

During the earlier period of our studies, as in 1916 and 1917, we supposed that it would be possible to isolate from various tissues a "motiline" which is not a biuret yielding peptid and which combines great power to stimulate plain muscle with the power to induce a more or less marked rise, not a shock-like fall, of blood pressure. In working with absolutely fresh mucosa of the small intestine, we naturally at once encountered the depressor constituent (3) of the secretin of Bayliss and Starling, and we accepted the prevailing opinion that we are dealing here with a substance histamine, of bacterial origin which is produced in the intestinal canal (4) by certain definite organisms from histidine, an amino-acid constantly present in the intestine. We believed for a time that it would be possible to remove completely this bacterial depressor substance, by means of the proper solvents, from a tissue such as the intestinal mucosa into which it was thought to have made its way by absorption only. The fact that we could prepare from the intestinal or gastric mucosa an extract, which, although still containing albumoses, stimulated the uterus in comparatively high dilutions (1: 1,000,000), and which appeared to be devoid of a depressor action on the blood pressure of the cat, supported us in this opinion. We found later that it

was necessary only to inject larger quantities of the preparation to induce a fall of blood pressure. Further experimentation with the freshly excised gastric mucosa of the dog and with freshly excised tissues in general, such as the liver, thyroid gland, and muscles, led us in time to the belief we now hold that the depressor substance cannot be separated from the plain muscle stimulant; that the stimulation of plain muscle and the depressor action belong in fact to one and the same substance wherever it be encountered. In a word, the "motilines," "peristaltic hormones," "vaso-dilatin" (Popielski), "histamine-like substances in the tissue" (Dale), etc., are all one and the same substance.

We have indicated above by the use of certain descriptive terms, as "motiline" and "peristaltic hormones," that some investigators¹ have studied extracts of animal organs mainly from the point of view of their ability to stimulate plain muscle. On the other hand, the terms, vaso-dilatin and dépressine² which have been widely used in recent years, indicate that others have studied such extracts more from the point of view of their powerful blood pressure lowering property. Popielski, who introduced the term "vaso-dilatin" to designate the depressor substance of Witte's peptone and tissue extracts in general, was so much impressed with its powerful action on blood pressure and also with its secretory power for the pancreas, liver, and other organs, that he interpreted the plain muscle stimulation of his extracts as the secondary result of the anemia induced by the low blood pressure (5). Dale and Laidlaw in a paper of fundamental importance (6) showed that various organ extracts and preparations, such as Witte's peptone, have a stimulant action on plain muscle as ex-

¹ See Weiland, Pflüger's Archiv, cxlvii, 171, 1912, for an extensive investigation in this field with much literature. Weiland is one of the writers who lay emphasis on the fact that organ extracts not only act as stimulants for plain muscle, but that they also depress the blood pressure. This author also made the interesting observation (apparently unaware of the earlier work of Dale and Barger and Dale and Laidlaw on histamine) that organ extracts may be entirely devoid of action on the blood pressure of anesthetized or decerebrate rabbits while having a marked depressor action on the blood pressure of the cats.

² The terms, urohypotensin and urohypertensin (Abelous and Bardier), used to designate certain as yet unidentified constituents of the urine that affect the blood pressure, may also be mentioned in this connection.

emplified by their action on the isolated uterus. These authors point out that the common group of effects, or symptom-complex, induced by Popielski's vaso-dilatin is, in almost all essential particulars, produced by histamine (β -iminazoly-ethylamine). They remark further,

In one respect the parallelism appears to break down. In the few experiments as yet made for the purpose, we have been unable to detect any action of injections of β -iminazoly-ethylamine on the coagulability of the blood. In other respects, however, the correspondence in action is so close as to suggest the presence in "peptone" and in various organ extracts of some substance at least related to β -iminazoly-ethylamine.

In a recent paper, Dale and his collaborators (7) have given us extensive and valuable pharmacological studies on the vaso-dilator action of histamine and on the general subject of histamine-shock. Among other notable findings, they have discovered that histamine and similarly active, as yet unidentified, substances in large doses induce an acute endothelial intoxication, with all the pathological consequences of this condition. In reference to poisons of animal or bacterial origin of similar action, Dale and Laidlaw write,

The action of histamine does not stand by itself, but represents in its most characteristic features a type of action common to a large group of poisonous substances of animal or bacterial origin. Substances having this type of action have been extracted from most of the organs of the body, though it is unlikely that histamine itself is among them, except in special cases such as that of the intestinal mucosa (Barger and Dale). It may be noted, however, that other substances of this class, e.g., bee poison or certain bacterial products, can produce either local inflammation and oedema or profound circulatory collapse, according to the method of application and the magnitude of dosage. A poison of the capillary endothelium seems to be the common factor in the action of all. This again brings them into relation with certain metallic poisons, such as arsenic and gold salts, which were long ago classed by Huebner as "capillary poisons," and which, when introduced in appropriate doses into the general circulation, produce a shock-like prostration. The shock-like symptoms which have occasionally followed the intra-

venous administration of salvarsan probably depend on an effect of this type—an acute endothelial intoxication. The existence of these points of community, in the action of substances so utterly unrelated chemically as histamine and certain metallic ions, forbids any assumption that the production of similar effects, by unknown constituents of some organ or tissue, indicates the presence therein of histamine itself, or of any substance chemically related to it.

A BRIEF OUTLINE OF EARLIER WORK ON HISTAMINE

This base which has in recent years acquired great significance for medical science was discovered in 1907 by Windaus and Vogt (8) who prepared it synthetically from iminazoly-propionic acid. In 1910, it was found by Barger and Dale (9), and independently and simultaneously by Kutscher (10) to be the most important constituent of ergot—that one of the ergot bases, in fact, which in minute doses produces tonic contractions of the uterus. This work was followed by the important investigation of the physiological properties of the base by Dale and Laidlaw (11). Yoshimura (12) in 1909 obtained a base from tamari-soya sauce to which he attributed the formula $C_6H_9N_3$, but which was undoubtedly histamine, $C_5H_9N_3$. In the following year (1910), working in the laboratory of food chemistry at Halle, this investigator (13) isolated appreciable quantities of histamine from putrid soy beans (0.18 grams from 1 kgm. of beans). At this time also, Ackermann (14) obtained a large yield of the base by submitting pure histidine hydrochloride to the action of putrefactive bacteria. This action consists merely in the removal by the bacteria of carbon dioxide from the histidine molecule, a reaction which is identical with the decarboxylation effected in an indirect manner in the synthesis of Windaus and Vogt. It was next isolated from the intestinal wall of the ox by Barger and Dale (15) in 1911 and was shown by them to be the depressor constituent of secretin mixtures, a constituent to which Bayliss and Starling had already called attention and which they had found to be extractable from fresh intestinal mucosa by means of alcohol. Mellanby and Twort (16) in 1912 then published a method for the isolation of a bacillus of the colon group from the contents of the intestines of

human beings and of other animals, which is capable of converting the non-toxic histidine into histamine. Believing that this work demonstrates the actual formation of histamine in the intestinal contents by bacteria, they wrote as follows:

In the alimentary canal of the guinea-pig, at least, and probably in that of most mammals, the bacillus capable of producing β -I. (histamine) from histidine is present from the duodenum downwards. It is legitimate, therefore, to assume that the presence of the histidine base, described by Barger and Dale, is due to bacterial decomposition going on in the intestine.

In a later paper (17), Mellanby again states,

That the constant formation of β -I (histamine), at least in the lower end of the intestine, is almost certain because of the presence of the amino-acid histidine and the necessary bacteria; a substance (β -I) of very potent physiological activities.....

Berthelot and Bertrand (18) in 1912 also isolated a bacillus from human intestinal contents which is able to change histidine into histamine, and later a second bacillus possessing this property was isolated by them from human faeces.

For the sake of completeness, we may add that W. F. Koch (19) found histamine present "in three urines out of six" from parathyroidectomized dogs, and that U. Suzuki and S. Ōtsuki (20) have shown that the base can be isolated from watery extracts of the muscle of the fish called *Maguro* (*Thynnus thunnus*). In explanation of its presence here, these authors say,

Inasmuch as *Maguro* meat contains much histidine, it is very well possible that some of this substance has been changed into histamine by decarboxylation. The question as to whether this transformation can occur in living tissues without the intervention of bacteria remains as yet undecided.

In view of the above citations it is not surprising that it should have become the accepted belief that whenever this toxic substance is encountered in the intestinal mucosa, its presence is fully accounted for by bacterial activity. Barger and Dale (21) were at

first inclined to regard the histamine isolated by them from the small intestines of the ox as a normal product of this tissue. Under the influence of the investigations cited above these authors appear to have modified their opinions as shown in the above citations from the recent paper (1919) of Dale and Laidlaw, and, as may be seen also in Barger's Monograph, *The Simpler Natural Bases* (p. 23), where this author says, "Its (histamine) formation in the intestinal wall is probably due to bacilli, isolated by Melanby and Twort and by Berthelot and Bertrand."

We, ourselves, were long hampered in our work by this prevalent opinion. We do not deny the possibility of the occurrence of histamine of bacterial origin in the intestinal contents, more especially in the lower part of the bowel, but when a "histamine-like substance" is found in equal amounts in both the fresh gastric and intestinal mucosa of the dog, when it is found in Witte's peptone and among the digestive split-products of proteins—that is to say, when it is present in all foods that contain proteids of animal or vegetable origin—it may well be asked whether any of this substance is really produced under normal conditions in our intestines.

It will be shown in the following pages, by a combination of chemical and pharmacological evidence, that the histamine-like substance of the materials just enumerated of organ extracts in general, more especially the hitherto unknown "active principle," or hormone, of the hypophysis cerebri (pituitrin or hypophysin) is in reality histamine (β -iminazolyl-ethylamine). In brief, it is our opinion that this substance makes its appearance wherever living protoplasm exists, or at least, wherever protoplasm is killed; in other words, that it arises wherever a true protein is even partially disrupted by enzymes, acids, or other hydrolytic agents.

HISTAMINE, THE PLAIN MUSCLE STIMULANT AND DEPRESSOR CON- STITUENT OF THE HYPOPHYSIS CEREBRI

Within the past twelve years, more or less purified decoctions or extracts of the posterior lobe of the pituitary body have acquired great importance in therapeutics, mainly because such prepara-

tions induce powerful contractions of the uterus (22) and intestines. The relation of the hypophysis to the complicated subject of internal secretion and to many pathological disturbances are of such prime importance that it is not surprising that this organ has occupied the attention of many investigators in widely differing fields of medicine and physiology. In 1913, the chemists of a large German chemical firm (23) announced the isolation, from the gland, of four active principles (24) in the form of sulphates. A mixture of the four crystalline sulphates was named Hypophysin and was stated by Fühner (25) to represent the physiological activity of the posterior lobe of the gland in respect to blood pressure, respiration, and uterine contractility, and the sum of the actions of the four crystalline fractions was stated to equal that of the undifferentiated hypophysis. Two years ago, Abel and Pincoffs submitted the claims of the Hoechst chemists to a critical examination, using for their work the hypophysis of the firm which was labelled a "sterile solution of 1:1,000 of the isolated active substances from the *glandula pituitaria*." The reader is referred to the paper of these authors for details of their chemical and pharmacological experiments, but the concluding paragraph of their paper may here be given.

The "Hypophysin" of the Farbwerke-Hoechst Company is not, as claimed for it, "a solution of the isolated active substances of the pituitary gland" but a mixture of albumoses (and possibly peptones) with varying and unknown amounts of active and inactive constituents of the gland. The albumoses present in "Hypophysin" account fully for the chemical reactions (such as the biuret and the Pauly reactions and the left-handed rotation) which are stated to characterize the pretended active principles. The albumoses as separated from the pituitary extracts are devoid of action upon the uterus. In view of the facts here presented it must be evident that the active principles of the hypophysis cerebri have not yet been isolated as chemical individuals.

An examination of the findings described in the following pages and a comparison of them with the detailed report given by Fühner of the physiological action of the four sulphates of the Hoechst chemists will make it evident, we think, that the assumed

four principles owed their action on the uterus, solely to the variable amounts of histamine included in their crystals, or adsorbed by, or chemically united with, the admixed proteoses.³

An earlier attempt of Engeland and Kutscher (26) to isolate an active principle from the gland calls for brief mention only. These writers obtained a base precipitable by phosphotungstic acid which had only a trifling action on blood pressure and uterine muscle and which differs widely in its chemical reactions attributed to it from the active principle as isolated by us. The "base" of Engeland and Kutscher evidently consisted of one or more of the many constituents of the gland which are precipitable by phosphotungstic acid, and owed its physiological activity entirely to a retention in small measure of the true plain muscle stimulant of the gland, β -iminazoly-ethylamine. Later authors have also not succeeded in separating a pure principle as may be seen from the perusal of a paper by M. Guggenheim and of the recent Monograph by Houssay (26 a) which contains a very complete bibliography of the subject.

METHOD OF ISOLATION

Before taking up the study of the hypophysis, we had learned from experiments with many other organ extracts that their depressor substance and plain muscle stimulant is taken up by hot chloroform from a dried and powdered preparation with which an excess of sodium carbonate had been incorporated before drying *in vacuo*. With this fact to guide us, a method of isolation was first tried out with 1 ounce of the dried substance of the entire pituitary body of the ox. Upon finding that we could extract a considerable proportion of the active principle from an alkaline powder with boiling chloroform, we applied our method as described below to 1 pound of the dry pituitary substance (entire gland).⁴

³ Now that the chief active principle of hypophysin has been shown by us to be histamine, it is evident that the proteoses of "Hypophysin" do not entirely account for the Pauly reaction, although accounting satisfactorily for the biuret reaction and the left-handed rotation. Histamine gives the Pauly reaction but not the biuret reaction and does not rotate the plane of polarized light.

⁴ Purchased from Armour and Company, Chicago.

The pound of finely powdered material was suspended in 8 litres of a 2 per cent solution of mercuric chloride in 0.8 per cent aqueous hydrochloric acid. The mixture was shaken vigorously at intervals during the day and allowed to stand overnight. Mercury was removed from the filtrate with hydrogen sulphide, and the mercury-free fluid was concentrated on the water bath under a revolving electric fan until it was quite free from H_2S , when sodium hydroxide was added until the fluid was only faintly acid to litmus. It was then concentrated on the water bath under the fan until its volume was reduced to approximately 150 cc. After removal of a little flocculent material, by filtration, a concentrated aqueous solution of phosphotungstic acid (1:1) and a little sulphuric acid were added until no further precipitate was produced. The precipitate was separated from the supernatant fluid by centrifugalization and was twice washed by the same procedure with a 1 per cent solution of H_2SO_4 containing a little phosphotungstic acid. The washed precipitate was then dissolved by repeatedly rubbing it up in a mortar with small quantities of a 10 per cent solution of NaOH , avoiding any considerable excess of alkali and in fact desisting from the attempt to dissolve the last portion of the precipitate. After filtering from the undissolved portion of the precipitate, the dissolved phosphotungstates were decomposed with the necessary quantity of warm $\text{Ba}(\text{OH})_2$ solution. A slight excess of barium was removed with H_2SO_4 and the neutral or faintly acid solution was concentrated on the water bath under the electric fan to a small volume. Alcohol (95 per cent) was added until inorganic salts and other alcohol-insoluble constituents of the mixture were precipitated. Two further extractions with the 95 per cent alcohol were made until only a grayish-white, physiologically inert, residue was left in the bowl. The alcoholic extracts were concentrated to a volume of about 45 cc. A solution was made containing 0.01 cc. of this liquid in each cubic centimeter of 0.8 per cent NaCl solution, and of this 0.5 cc. was injected intravenously into a cat weighing 2.35 kgm. induced the characteristic fall of blood pressure which is produced by the injection of about 0.25 mgm. of β -I. In accordance with this estimate our mercuric chloride extract of

the pound of dried pituitary substance (one extraction only) contained 225 mgm. of the active substance.

In laboratory parlance, we are accustomed to speak of that quantity of the pressor principle necessary to induce a fall of pressure in a cat weighing 3 to 4 kgm. equal to that caused by 0.05 mgm. β -I. as one "dose" or one "large dose." As a matter of fact solutions may be quite accurately standardized in this way and it is easy to trace one's losses in various bulky precipitates by means of the blood pressure test.

To the concentrated fluid, powdered Na_2CO_3 in excess was added, the mixture was transferred to bowls and these were placed in vacuum desiccators charged with H_2SO_4 and left there until the alkaline residues were dry or nearly so. They were then extracted four times in succession with warm 95 per cent alcohol. The alcohol of the combined extracts was driven off on the water bath, and the residue was repeatedly extracted with cold absolute alcohol (six extractions). The insoluble residue left by the 95 per cent alcohol was almost entirely inactive, while the residue left undissolved by the absolute alcohol contained 1600 "small doses," a "dose" in this case being approximately equal to 0.02 mgm. β -I. After driving off the absolute alcohol, the residue was taken up in a small volume of water, a hot saturated solution of HgCl_2 was added, the mixture was then cooled on ice and a cold saturated solution of HgCl_2 was added, as long as crystals could be induced to appear on rubbing the inside of the containing beaker with a glass rod. The mercuric chloride compound thus thrown out was collected, washed with cold saturated HgCl_2 solution and decomposed with H_2S . To the filtrate from the HgCl_2 precipitate, we added sodium hydroxide until no further precipitate of a flocculent nature was produced. This precipitate was collected by centrifuging, washed, and likewise decomposed with H_2S .

We were still experimenting with new procedures at this time and in consequence our active substance was divided at this point. Blood pressure tests made with both mercury-free solutions showed that both precipitates had taken down the active principle. As about twice as much was carried down in flocculent alkaline mercury precipitate, we shall here describe the method

used with this fraction, merely stating that the material obtained from the other precipitate was also worked up by more or less similar methods to an end point when a crystalline picrate could be prepared. This picrate was, however, less pure (80 per cent pure) than that which was obtained from the main fraction. We estimated, by means of the blood pressure method, that this impure picrate contained about 10 mgm. of the active base.

To return to the liquid obtained by decomposing the alkaline HgCl_2 precipitate. This was concentrated in the usual way to a small volume, alcohol was added, and the solution again concentrated, and this was repeated until the residue was practically free of hydrochloric acid. It was now taken up in a little water, saturated solution of mercuric chloride was added, and the mixture was kept on ice until no further precipitate was obtained. The filtrate was freed from mercury with H_2S , the solution concentrated to a small volume, freed from excess of HCl as before, an excess of powdered Na_2CO_3 was added, and the mixture was concentrated to dryness in a vacuum desiccator. The dry residue was reduced as nearly as possible to a powder and repeatedly extracted with small quantities of boiling chloroform. (On allowing the CHCl_3 solution to stand overnight, groups of prismatic crystals settle out on the sides and bottom of the containing flask. From these crystals a picrate is readily made, which is slightly active because of admixture with the active principle. On recrystallization, the picrate loses its depressor action entirely. It gives the Pauly reaction and we suspect that we are here dealing with a little histidine which has gone into the hot HCl_3 in company with the histamine and perhaps other substances which have rendered it soluble in this reagent.)

After driving off the CHCl_3 , the residue was exhausted several times with a small quantity of absolute alcohol to which a little 25 per cent sulphuric acid had been added. The acidulated alcoholic extract was concentrated somewhat and was then allowed to fall, drop by drop, into a considerable volume (relatively) of a mixture of one volume of absolute alcohol to two volumes of dry ether. An amorphous and highly active sulphate is thrown out

in this procedure while the alcohol-ether fluid contains only a negligible quantity of the base. The precipitated sulphate is dissolved in as little water as possible, a concentrated solution of gold chloride and a drop or two of hydrochloric acid solution are added. The solution was concentrated on the water bath under a fan until crystals began to make their appearance on the surface of the liquid.

We may here remark that when working with other tissues we have usually found it necessary to remove a resinous precipitate which appears at this point on the addition of gold chloride, especially when the solution is dilute and only a little acidulated. In case a resinous precipitate appears on diluting the gold solution with water, it is well to allow this to settle and then to remove the clear supernatant liquid. This is then concentrated on the water bath to a point at which crystals of the gold compound begin to appear while the solution is still hot. We have also used platinum chloride at this point, but gold chloride has been more satisfactory on our hands, probably because it forms fewer water insoluble, or relatively insoluble, compounds with the impurities still present in the chloroform extract.

The gold chloride compound is washed in the bowl with a small quantity of ice water by means of a pipette with a capillary point and decomposed with H_2S . From the gold-free filtrate, concentrated, and freed entirely from hydrochloric acid, the crystalline dipicrate is made by the addition of a saturated solution of sodium picrate.

The yield of picrate⁵ $C_5H_9N_3(C_6H_3O_7N_3)_2$, from the alkaline mercuric chloride fraction as outlined above was 0.018 gram. It was found to be very active, and very nearly pure, as compared with β -I. in its action on the blood pressure and uterus. Its melting point was 236° to 237° , whereas that of pure β -I. picrate is 239° to 240° , or even a little higher according to the rate of heating. The salt was now twice crystallized from hot water and about 5 mgm. of a fraction was obtained which gave the Pauly reaction with great intensity and melted to a dark fluid with

⁵ The dipicrate, $C_5H_9N_3(C_6H_3O_7N_3)_2$ is meant whenever we speak of the picrate of "our base" or of histamine.

evolution of gas bubbles at 242° to 243°C. on rather rapid heating. A specimen of β -I. picrate prepared by ourselves and twice crystallized when heated at about the same rate, melted and decomposed at 241° to 242°C. The two compounds are quite identical in respect to crystalline form, melting point, solubility, and physiological behavior. One has only to recall the procedures employed in the method of isolation to find further proofs of identity. Thus, our principle is precipitable by phosphotungstic acid, by mercuric chloride plus the hydroxide of an alkali metal, and yields a crystalline aurichloride and platinichloride. As a free base, it is soluble in water, alcohol, and in hot chloroform, and practically insoluble in dry ether. β -iminazolyethylamine behaves in an identical manner toward all of the tests named.

Equally striking is the identity of the two substances in respect to their physiological activities. The following tracing (fig. 1) shows that our final picrate (melting point 242°C.) lowers the arterial pressure weight for weight in exactly the same degree as pure histamin picrate, $C_6H_9N_3(C_6H_3O_7N_3)_2$. Figure 2 demonstrates that the two picrates act in the same way and with equal strength as uterine stimulants. Figure 3 shows the effect on the rabbits' blood pressure of a chloroform extract from which the pure picrates were derived.

It may be noted at this point that the dipicrate of histamine (and hence of our substance likewise) contains slightly less than one-fifth of the free or active base in its molecule as the two molecules of picric acid constitute four-fifths of the weight of the molecule. The strength of the solution actually used, therefore, in the experiments on the guinea-pig's uterus is in reality 1:5,000,000 instead of 1:1,000,000 and this indeed is a higher concentration than is necessary. It may be in place here to note that if one is employing very high dilutions of a histamine salt in comparative uterine tests, very great accuracy in weighing as also in the use of calibrated burettes or measuring flasks is to be observed. At dilutions of 1:30 or 50 millions, any errors in the above particulars are so far magnified that two specimens may appear to be of different strength when they are in reality of the

same strength. Indeed at these high dilutions the balance error, in weighing out say 3 mgm. of pure β -I. picrate as against 25 mgm. from the same lot, will manifest itself at very high dilutions, that is to say at dilutions whose action approaches the

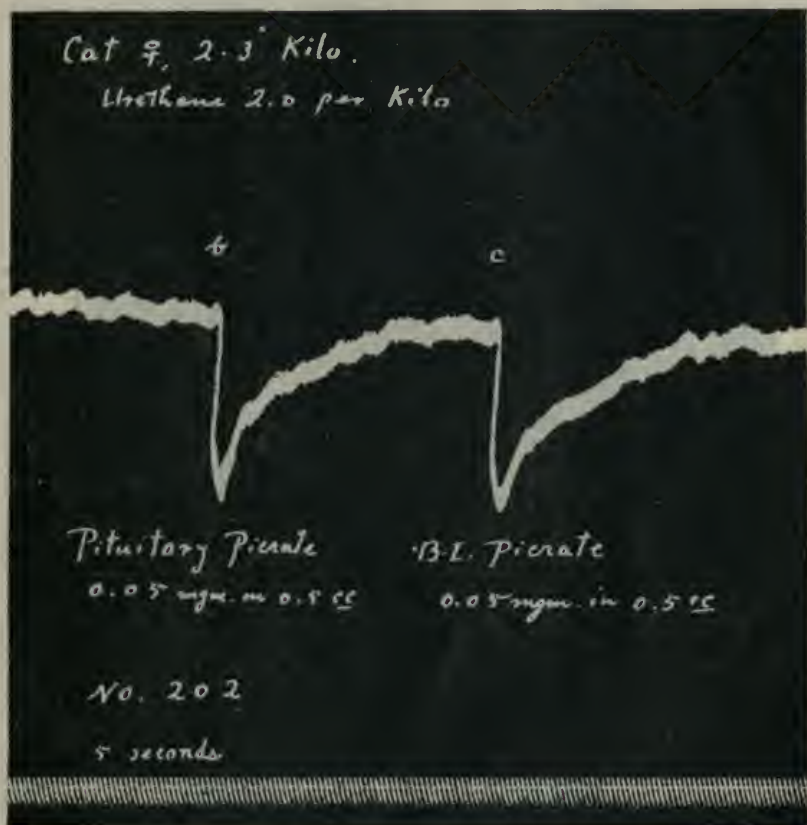


FIG. 1. COMPARATIVE ACTIVITY OF THE PICRATE PREPARED FROM THE PITUITARY GLAND AND THE PICRATE OF HISTAMINE ON THE BLOOD PRESSURE OF THE CAT

threshold value of stimulation for the uterine muscle. In standardizing an unknown preparation, therefore, these points must be borne in mind.

We have found that the depressor action on the cat is well

adapted for comparative tests, as already intimated in the foregoing description of our method of isolation from the hypophysis. In the cat anesthetized with urethane, the response to equal doses continues to be quantitative for many hours. Doses corresponding at most to 0.05 mgm. of the free base for a cat of average size should be used. With such doses, or with those still smaller, differences in the purity of two preparations are

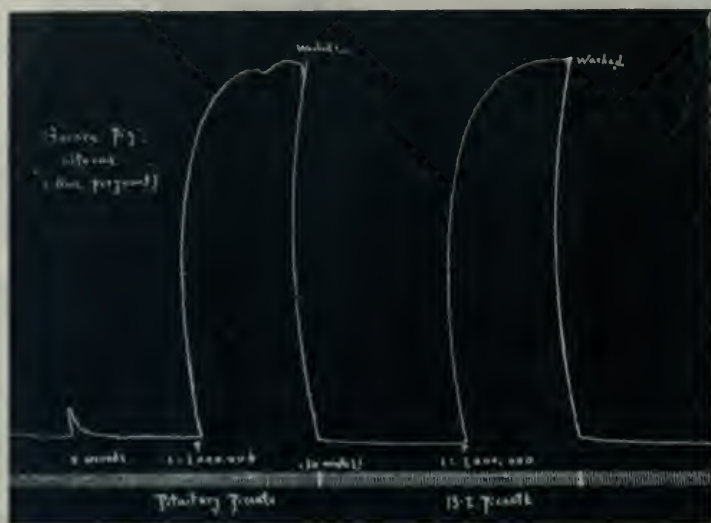


FIG. 2. COMPARATIVE ACTIVITY OF THE PICRATE PREPARED FROM THE PITUITARY GLAND AND THE PICRATE OF HISTAMINE ON THE UTERUS OF THE VIRGIN GUINEA-PIG

A half horn was suspended in 30 cc. Tyrode's solution. In all experiments the ratio of the lever arms is 1:7 unless otherwise stated. (Reduced to $\frac{1}{2}$).

readily shown on the blood pressure tracing. Naturally here, as in the comparative tests on the uterus, great care must be exercised in weighing and in the use of volumetric apparatus. As in the employment of the uterus or the intestine as a test object, so here also the use of strong solutions which cause a prolonged fall of blood pressure is to be avoided in making comparisons.

We may here give the results of our estimates of the yield of

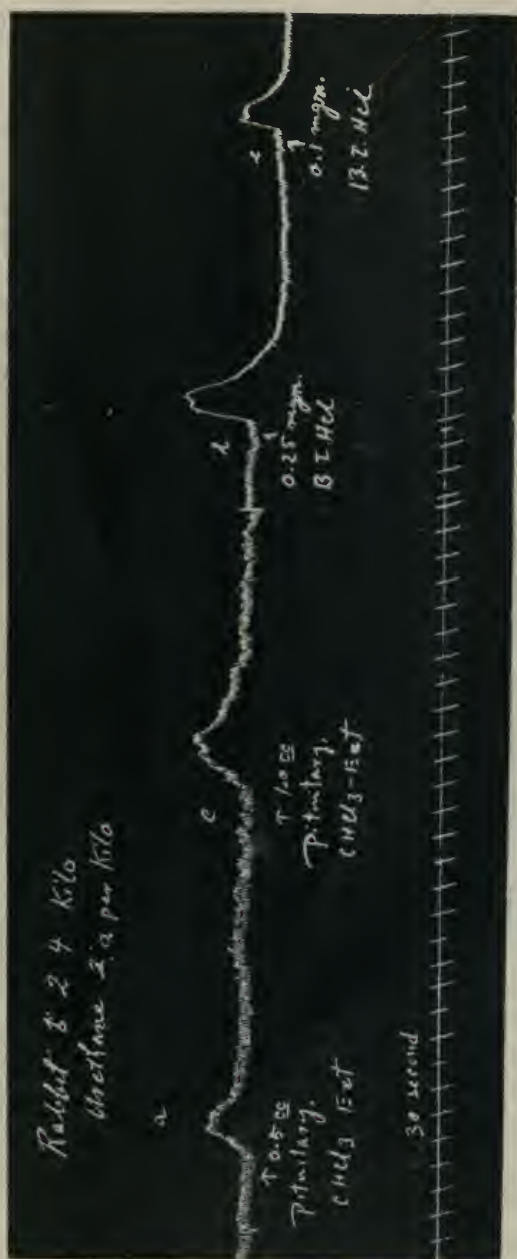


FIG. 3. ACTION OF A CHLOROFORM EXTRACT FROM THE PITUITARY GLAND ON THE BLOOD PRESSURE OF THE RABBIT
COMPARED WITH THAT OF HISTAMIN HYDROCHLORIDE

final product. It has been stated that we judged that our mercury-free extract of the pound of dried pituitary substance contained approximately 225 mgm. of histamine.⁶ The particular precipitate, obtained with mercuric chloride and sodium hydroxide, which was worked up to the stage of a nearly pure picrate, yielded 18 mgm. of this salt as already stated. We calculate, on the basis of blood pressure estimations made with other more or less impure sulphates or picrates which were recovered from various wash fluids and precipitates, that these recovered fractions contained 38 mgm. of the base. Naturally the greater part of this recovered material was again lost in trying out new methods and in tests which prepared us for the final identification of the base. We had in hand, therefore, about 56 mgm. of a more or less pure product. Even though this amount had been at our disposal in a single portion, further purification would surely have reduced it to about 25 mgm. With our present methods, we can not expect to obtain much more than a 10 per cent yield of a pure product from the pituitary gland.

HISTAMINE FROM THE GASTRIC AND INTESTINAL MUCOSA OF THE DOG

A large dog weighing 20.14 kgm. was deprived of all food except water for the four days preceding the experiment. The animal was killed by bleeding from the carotid artery and later the abdominal aorta was severed at the diaphragm. The stomach and small intestines were removed immediately, opened, and washed thoroughly, first under running water (mucous and adherent bile being rubbed away with the fingers), and then the cleaning was continued in large bowls of 0.8 per cent NaCl solution. The surfaces were then dried by pressure against large sheets of filter paper, after which the entire mucous layer of both stomach and intestines was scraped away with sharp edged pieces of glass. The scrapings weighed 242 grams and the discarded muscular part of both viscera weighed 335 grams. To

⁶ This is the amount obtained in *one* extraction. Further extractions would no doubt have yielded a little more of the base.

the scrapings we added 1210 cc. of a 2 per cent aqueous solution of HgCl_2 , containing 0.8 per cent of HCl . The mixture was well shaken at intervals during the day and larger fragments of mucosa were broken into smaller pieces by mechanical means. The next day and the day following, the mixture was filtered at the pump on flat porcelain funnels until 1335 cc. of a more or less clear filtrate was obtained. A part of the filtrate was now used for albumose determinations and these and peptones were found to be present in such very large amount that it became evident that hydrolytic (HCl) or enzymatic processes had been steadily at work during the three days. Bacterial action is certainly excluded (2 per cent HgCl_2).

The solution stood for four months in the dark because of pressure of other work, after which time 730 cc. of it were treated as follows: Mercury was removed with H_2S , the filtrate concentrated and freed from excess of HCl , and then precipitated to the limit with an aqueous solution of phosphotungstic acid (1:1). The phosphotungstic acid precipitate was twice washed at the centrifuge with an acidulated, very dilute solution of phosphotungstic acid and was then dissolved to the extent to which this could easily be done by the cautious addition of 10 per cent NaOH and decomposed with $\text{Ba}(\text{OH})_2$ as described in the section on the pituitary gland. The neutralized filtrate was concentrated to dryness (vacuum desiccator at the last) and then exhausted five times with absolute alcohol, containing a trace of sulphuric acid, the alcohol driven off, the residue taken up in water, and shaken out three times with cold chloroform in a separator funnel in order to remove physiologically inert substances of lipoid character. The aqueous solution was then concentrated to a small volume, saturated with powdered sodium carbonate and dried *in vacuo*. The dry residue was next extracted repeatedly with hot chloroform. On evaporating the solvent on the water bath, it was found that the chloroform had extracted a small amount of basic material which remained in the bowl as whitish sticky lumps or droplets. This material was found to stimulate the isolated uterus in minute amounts. The droplets were now dissolved in a little water acidulated with sulphuric acid and the

solution was freed from a little flocculent material by filtration. The solution was concentrated to a very small volume (about 0.5 cc.) which was transferred to the bottom of a short test tube. A little absolute ethyl alcohol was then stratified over the solution. Above this absolute methyl alcohol was stratified until the tube was nearly full. It was set aside for five days when a quantity of prismatic crystals was found deposited in the bottom of the tube. The crystals were washed with a little cold methyl alcohol, dissolved in a little water and changed into the crystalline aurichloride. A small resinous lump which was deposited in the mother liquor along with the crystalline aurichloride was easily separated from the crystals. The aurichloride was dissolved in water and freed from gold with H_2S . The resulting very soluble hydrochloride was allowed to crystallize *in vacuo* when it was seen to be quite identical in respect to its physical and chemical properties with the corresponding salt of histamine, $\text{C}_6\text{H}_9\text{N}_3 \cdot 2\text{HCl}$.

Comparative tests, on blood pressure and on the guinea-pig's uterus, were made, and it was found that the hydrochloride differed in hardly perceptible degree from the corresponding salt of synthetically prepared histamine.

In order to furnish additional evidence that we were dealing with histamine, the hydrochloride was dissolved in water and sodium picrate solution was added. Immediately the slightly soluble crystalline dipicrate fell out. This was recrystallized twice and matched against pure recrystallized histamine dipicrate, weight for weight, on blood pressure and uterus tests, the results of which are given in figures 3 and 4. The melting point of the picrate was found to be 238° to 239°C . on fairly rapid heating, and that of β -I. picrate in a similar capillary tube was found to be the same.

The physiological and chemical proofs given allow no escape from the conclusion that here as in the case of the pituitary, the substance isolated by us is no other than histamine. It may be questioned whether an appreciable amount of the histamine isolated in the foregoing experiment is of bacterial origin. The conditions of the experiment were such that the greater part of the base, if not all of it, must have been produced by non-bacterial hydrolytic (autolytic) processes.

The substance was also isolated in the form of a slightly impure picrate from the upper portion, 3 or 4 feet in length, of fresh pig's intestine. The intestines were brought, still warm, from a slaughter house in close proximity to the laboratory, and the scrapings were worked up immediately without being subjected to a prolonged hydrolysis as was the case with the dog's intestine. And here also it may be questioned whether any of the base was present in the preformed state, except in so far as it was present in the mucosa as a constituent absorbed from the animal's food.

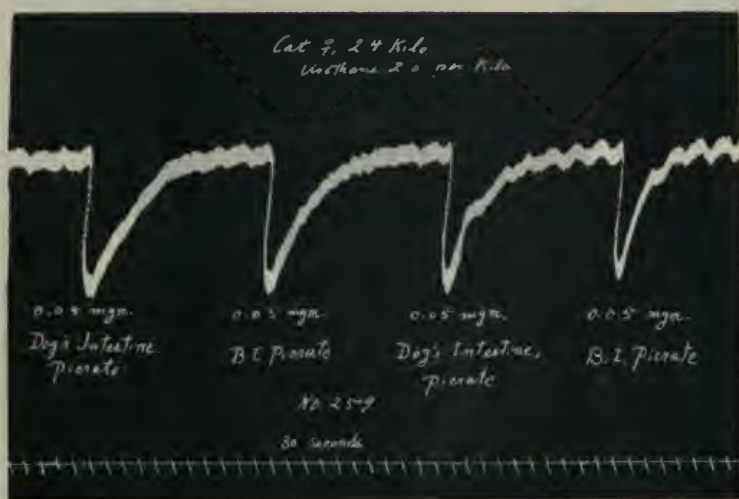


FIG. 4. COMPARATIVE ACTIVITY OF A PICRATE, PREPARED FROM THE INTESTINAL AND GASTRIC MUCOSA OF THE DOG, ON THE BLOOD PRESSURE OF THE CAT. (Reduced to $\frac{2}{3}$)

In regard to the yield of histamine in the above experiment with the gastric and intestinal mucosa of the dog, we are able at the moment to give only approximate figures, although it is possible as already stated to make estimates of this sort with considerable accuracy. Our blood pressure tracings show that the mercury-free solution obtained from the 730 cc. of extract employed contained 1460 doses of histamine of approximately the value of $\frac{1}{15}$ mg. per dose. This would show a content of 100 mgm. in round numbers. We have already stated that we judge

that we obtained 15 mgm. of pure histamine hydrochloride from the extract used, indicating a 15 per cent yield of active principle. It must be noted that we do not by any means completely extract the histamine from any of the tissues examined and that there are numerous unavoidable losses at almost every step in our processes. It is apparent, however, that the enzymatic (hydrolytic) breaking down of a tissue, such as the gastric and intestinal mucosa, may give rise to large quantities of this toxic substance.



FIG. 5. COMPARATIVE ACTIVITY OF THE TWO PICRATES USED FOR FIGURE 4 ON THE UTERUS OF THE VIRGIN GUINEA-PIG

A half horn was suspended in 30 cc. Tyrode's solution. (Reduced to $\frac{1}{2}$)

HISTAMINE FROM THE LIVER

Because of its connection with the absorbing surfaces of the stomach and intestines, we should expect to find histamine in the freshly excised liver. We are, however, quite in the dark as to what extent this organ alters histamine and renders it physiologically inert. It was our purpose here, not so much to isolate the substance from the liver in a pure state as to prove with a reasonable degree of certainty that it exists in the fresh organ. Our investigation has now, very naturally, included the study of so many tissues that it has become impossible for us to isolate the histamine in the pure state in all cases in time to include the results in the present paper.

Method in outline. Four hundred and sixty-five grams of liver were removed from a dog bled to death under ether anesthesia, cut into small

pieces and boiled in its own juices with steam in a "double boiler" for fifteen minutes. The cooked tissue was then rubbed up in a mortar with the 2 per cent mercurial solution, as already described for other tissues. Five hundred cubic centimeters of the mercurial solution were employed in all, and in addition, 50 grams of powdered HgCl_2 were added to the mixture. On the following day, 350 cc. of the filtrate were treated in the usual manner, phosphotungstic acid was used as before, and the resulting sulphates were repeatedly extracted with boiling absolute alcohol. The material extracted by the alcohol was dissolved in water, picric acid in slight excess was added, and the flask was set aside for the night. The conditions of the precipitation with picric acid here were such that only a very small amount of histamine was carried down with the mass of foreign picrates, while most of the base was found in the filtrate. The filtrate was freed from picric acid with ether, made alkaline with Na_2CO_3 , concentrated to a small volume, dried *in vacuo* over H_2SO_4 , and extracted repeatedly with boiling chloroform. On evaporating the chloroform a small amount of physiologically very active material was left. This was dissolved in 10 cc. of water and neutralized with sulphuric acid.

Yield of histamine. According to our blood pressure estimations, as made on the cat, the chloroform extract contained 17.5 to 18 mgm. of histamine. The 350 cc. used for the extraction of this amount contained fully 50 mgm. of histamine. It is apparent, therefore, that the above simple procedure gave us a yield of 36 per cent of a more or less impure chloroform residue. Naturally, we should have been obliged to make use of a number of other processes in order to obtain an absolutely pure end product, and the final yield as in all other cases would have been considerably smaller, 15 per cent or thereabouts, at the most.

The accompanying figure (fig. 6. A and B) shows at "A" the effect on the blood pressure of injecting one-fiftieth of the solution of the chloroform extract into a cat and at "B" the effect of injecting one-fiftieth of the solution into a rabbit. Here the paradoxical rise, so characteristic of the action of histamine, in this animal is quite apparent. Figure 7 shows the effect of a minute amount of the chloroform extract on the guinea pig's uterus. When the chemical evidence, especially the solubility of the substance in chloroform and its basic character are taken

together with the above physiological effects, notably the paradoxical rise of arterial pressure in the rabbit, it seems certain that as in the intestinal mucosa and the pituitary we are here also dealing with histamine.

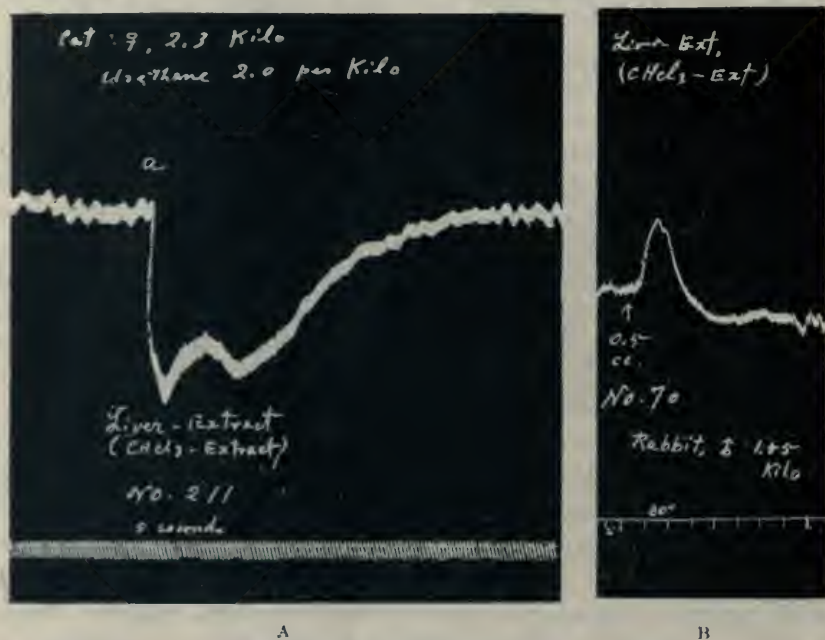


FIG. 6. SHOWS THE EFFECT OF A CHLOROFORM EXTRACT FROM DOG'S LIVER ON THE BLOOD PRESSURE OF THE CAT (A) AND OF THE RABBIT (B)

HISTAMINE FROM STRIATED MUSCLE

Method. Thirteen hundred grams of muscle tissue removed from the hind legs of a large dog immediately after bleeding the animal and transfusing with Locke's solution (this in order to obtain blood for other purposes) were ground up in a sausage machine and immediately cooked by steam for fifteen minutes in its own juices in a "double boiler." The cooked muscle was then ground up with a 2 per cent solution of HgCl₂ in 8 per cent HCl, 5.2 litres of this solution being used for the above weight of muscle. The method of treatment from this

point was practically like that already described for other tissues, that is to say, phosphotungstic acid, mercuric chloride alone and in combination with sodium hydroxide, gold chloride and sodium picrate were employed as precipitating agents, while alcohol, chloroform and water were used as solvents. Details in respect to the employment of these methods may be omitted.

In the case now under consideration we encountered difficulties because we supposed that we could shorten our labors by using picric acid or sodium picrate at an earlier stage than in some of our earlier work. There are quite a number of constituents of

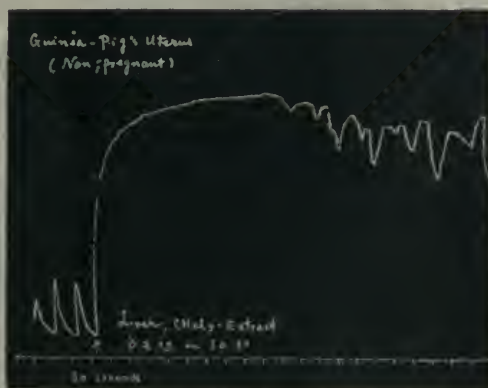


FIG. 7. SHOWS THE EFFECT OF A CHLOROFORM EXTRACT FROM DOG'S LIVER ON THE UTERUS OF A VIRGIN GUINEA-PIG

A half horn was suspended in 30 cc. of Tyrode's solution. (Reduced to $\frac{2}{3}$)

animal tissues, some of them probably as yet unidentified, which form more or less difficultly water-soluble picrates. These substances mutually affect each others solubility in water and alcohol, and it becomes almost impossible to separate their picrates by fractional crystallization, except in cases where one of the number greatly predominates. We have, therefore, learned to avoid the use of this reagent in the earlier steps of the isolation of a histamine salt, and now employ it only as a final precipitant for salts which are already pure.

Having become involved in difficulties with our picrates, as

just stated, we changed what was left of them into sulphates, calling the product an "impure sulphate." Figures 8, 9 and 10

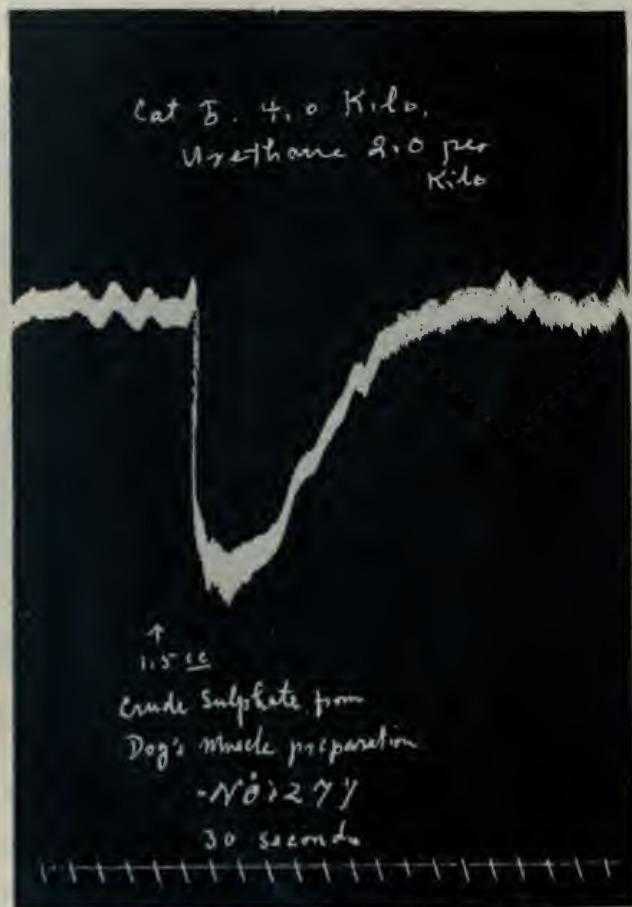


FIG. 8. SHOWS THE EFFECT OF AN IMPURE SULPHATE PREPARED FROM DOG'S MUSCLE ON THE BLOOD PRESSURE OF THE CAT

give the results of physiological tests made with this sulphate as obtained from the skeletal muscle of the dog.

HISTAMINE, A CONSTITUENT OF EREPTON

Erepton, which was introduced into dietary therapeutics by Abderhalden, is stated to consist only of the completely digested proteins of meat, that is to say, the product contains only amino acids and a-biuret peptides.

We have had occasion several times in the past two years to give large dogs as much as 150 grams of this preparation at one time, mixing it with a little butter and pure starch, after the dogs

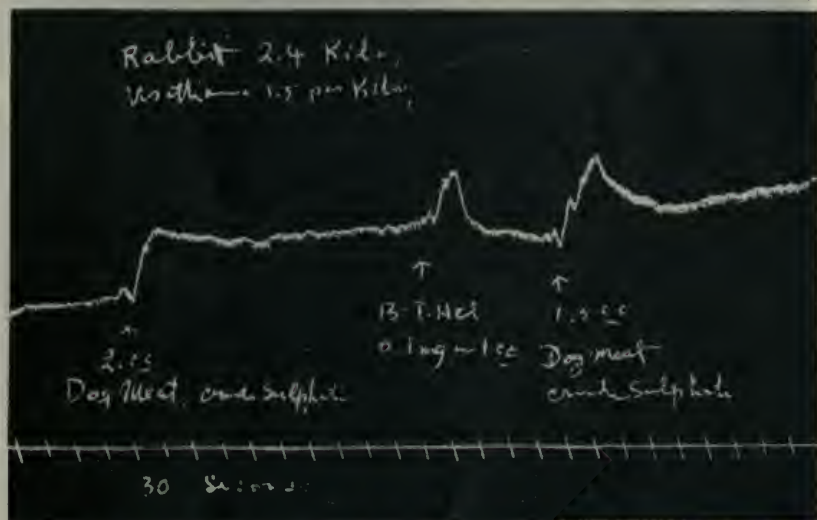


FIG. 9. SHOWS THE EFFECT OF AN IMPURE SULPHATE PREPARED FROM DOG'S MUSCLE ON THE BLOOD PRESSURE OF THE RABBIT

had previously gone without solid food for three days. The animal would generally reject the meal shortly after eating it greedily, but would soon again eat the vomited mass and then lie down quietly, apparently greatly contented. We infer that this emetic action is due to the stimulation of the musculature of the stomach by the considerable amounts of histamine contained in the erepton. With the exception of the vomiting no symptoms of any kind were noted. We, ourselves, have long known, and we suppose that other observers also are aware of the fact, that

erepton, which is quite free from peptones and albumoses, is very toxic when injected into the circulation, fully as much if not more so than Witte's peptone. The cause of the toxic, or shock-like action, is the same in both cases as we shall attempt to show, being due in both instances to histamine. On injecting 0.25 gram of erepton dissolved in 0.5 cc. of water into the saphenous vein of a dog, weighing 7.5 kgm., we found that the blood pressure fell suddenly from a level of 182 mm. of mercury to

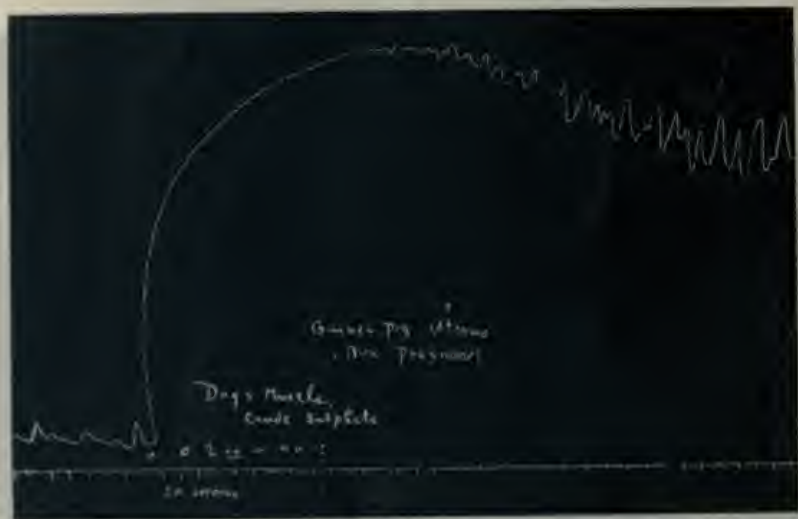


FIG. 10. SHOWS THE EFFECT OF AN IMPURE SULPHATE PREPARED FROM DOG'S MUSCLE ON THE UTERUS OF THE VIRGIN GUINEA-PIG

One entire horn of the uterus was used. Bath volume 50 cc. (Reduced to $\frac{1}{2}$)

85 min., a drop of more than 50 per cent, and that it required six and one-half minutes to return to the normal. Numerous experiments were made two years ago with the same result, all proving that erepton contains a very powerful depressor substance.

The same methods of isolation were used as with the muscle, and we may state that erepton is much more difficult to investigate chemically than fresh tissues, such as muscle or the liver because of the preponderating amount of amino-acids present.

We made the same mistake here as in working with muscle and used picric acid too soon. Not being able to prepare a pure salt in time for this paper, we content ourselves with giving the following figures (figs. 11 and 12) in which the action of solutions

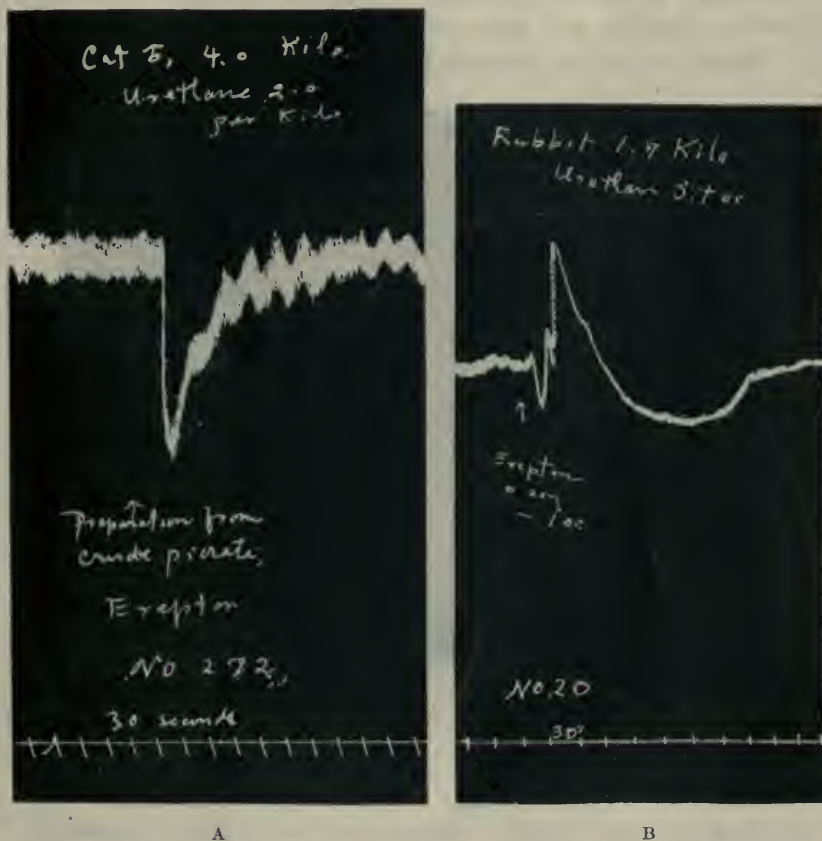


FIG. 11. (A) SHOWS THE EFFECT OF AN IMPURE PICRATE PREPARED FROM EREPTON ON THE BLOOD PRESSURE OF THE CAT. (B) SHOWS THE EFFECT OF 0.25 GRAM EREPTON ON THE BLOOD PRESSURE OF THE RABBIT

prepared from an impure picrate is shown on blood pressure and on the virgin guinea-pig's uterus. The picrate was dissolved in water, hydrochloric acid was added, the solution was shaken with ether until it was free from picric acid, neutralized, and used for

the experiments which are illustrated in figures 11, A and 12. In figure 11 B is shown the rise of blood pressure, so characteristic of histamine, in the rabbit, the quantity injected being the same as that which always causes a great drop of blood pressure in the dog. Here as in the case of dog's muscle, the two lines of evidence, chemical and physiological, justify us in the belief that the "shock poison" of erepton is histamine.

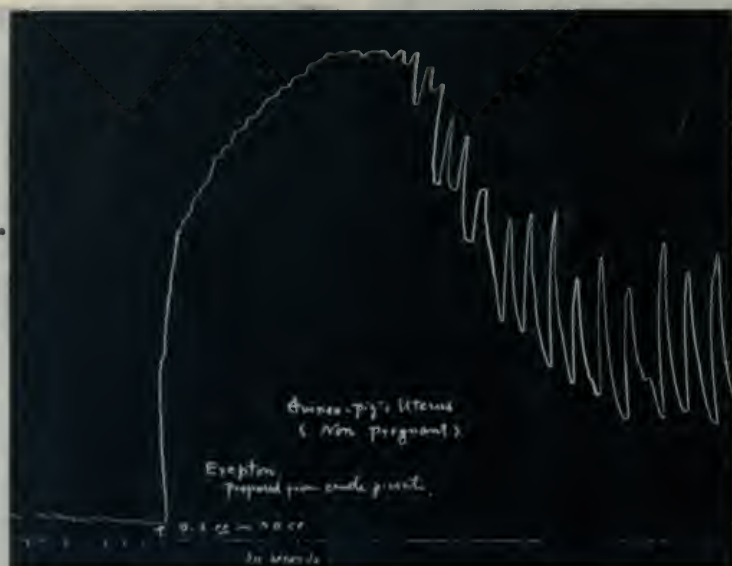


FIG. 12. SHOWS THE EFFECT OF A PREPARATION FROM THE IMPURE PICRATE FROM EREPTON ON THE UTERUS OF THE VIRGIN GUINEA-PIG

One entire horn was suspended in 50 cc. of Tyrode's solution. (Reduced to $\frac{1}{2}$)

HISTAMINE, THE TOXIC CONSTITUENT OF WITTE'S PEPTONE

Since Schmidt-Mülheim (27) first made his fundamental experiments with Witte's peptone in Carl Ludwig's laboratory, in 1880, and showed that a "peptone" prepared from this mixture of proteoses is capable of producing, when injected into the circulation, a fatal lowering of arterial pressure with terminal convulsions, Witte's preparation has been the subject of many investigations. Brieger (28) digested moist fibrin with an extract

of the pig's gastric mucosa for twenty-four hours at body temperature and was able to show that a poisonous substance makes its appearance among the products of digestion. He named the hypothetical substance peptotoxin and stated that it is also formed when proteins, such as fibrin and casein, or tissues, such as the brain, liver, and striated muscle, are allowed to undergo putrefaction. A comparison of the properties of our substance with the numerous, and in part mutually contradictory, reactions of Brieger's peptotoxin makes it quite clear that his toxin consisted merely of a mixture of an unknown number of substances with a very small proportion of the true poison. Salkowski (29), whose position as the director of the Chemical Division of Virchow's Pathological Institute and whose reputation as a biological chemist gave weight to his opinions, subjected Brieger's work to a critical analysis of destructive nature and arrived at the conclusion that Brieger's peptotoxin has no existence except as it may have its origin in bacterial activity. According to Salkowski, albumoses and peptones are inherently toxic substances and no poisonous principle whatever can be extracted from them. These proteoses, he says, do not poison us because they are not absorbed as such (Hofmeister), but are transformed into body-proteins in the intestinal and gastric walls.

Twenty years later Popielski (30) revived the doctrine of the separable toxin in Witte's peptone. This toxin, as he demonstrated in numerous experiments has certain physiological characteristics—depressor action on blood pressure and secretory action on the pancreas and other glands, and these activities, he showed, are manifested by organ extracts in general. As is well known this author gave the name vaso-dilatin to the assumed ubiquitous principle. By a simple procedure (solution of Witte's peptone in a very little water and precipitation with hot absolute alcohol, and repetition of this process) he was able to secure his vaso-dilation in concentrated form as compared with the original peptone. Naturally, this procedure could not lead to even approximate purity in the chemical sense. Later Popielski and Panek (31) (32) prepared specimens of vaso-dilatin by the use of cadmium chloride, mercuric chloride, and alcohol, which no

longer gave the biuret reaction, which lowered blood pressure, and which caused an increased flow of pancreatic juice. This method also could not yield anything but an impure final product, but Popielski was nevertheless justified in concluding that his vaso-dilatin is a product of the digestion of proteids, equally with pep-



FIG. 13. SHOWS THE EFFECT OF A CHLOROFORM EXTRACT FROM WITTE'S PEPTONE ON THE BLOOD PRESSURE OF THE CAT

tones and albumoses, and entirely separable from the latter. In regard to certain physiological properties of his vaso-dilatin, this author, as already stated, held quite erroneous views. Thus, he maintained that its action on plain muscle is a secondary effect brought on by the low blood pressure and the resulting

accumulation of CO_2 in the tissues. It has already been stated that Dale and Laidlaw were the first to show that organ extracts and Witte's peptone behave like histamine in these respects as well as in their action on blood pressure and glandular organs.

We have not thought it worth while for the present to spend much time on the isolation of histamine from Witte's peptone, partly for the reason that we have already demonstrated its presence in erepton which is only a more completely digested Witte's peptone, and partly because the senior author (A) pur-

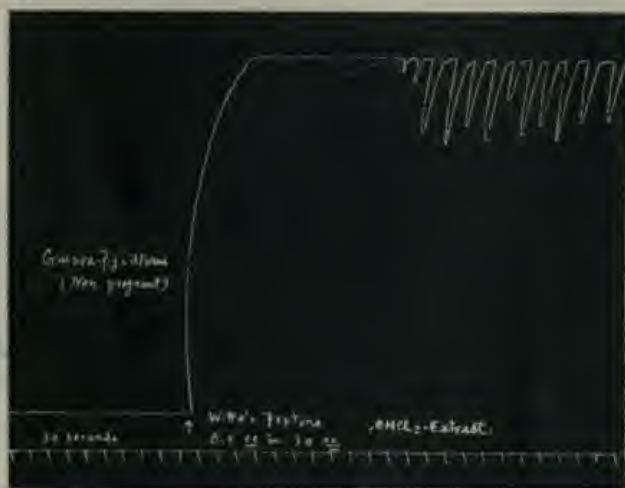


FIG. 14. SHOWS THE EFFECT OF A CHLOROFORM EXTRACT FROM WITTE'S PEPTONE ON THE UTERUS OF THE VIRGIN GUINEA-PIG

One entire horn was suspended in 50 cc. of Tyrode's solution. (Reduced to $\frac{2}{3}$)

poses during the coming winter to isolate it as a pure individual from among the hydrolytic split-products of pure proteins which have been subjected to the action of acids or alkalis. In a later part of this paper it will be shown that we may assume with practical certainty that this poison (or one of its homologues) is present among such split-products. Once shown to be derivable from proteins by chemical methods, further isolation, except in quite special cases, becomes superfluous.

The following facts fit in well with what has been demonstrated

in regard to the presence of histamine in various tissues and make it practically certain that this base is indeed the toxic agent of Witte's peptone. To 364.5 grams of Witte's peptone, we added 500 cc. of absolute alcohol and 10 cc. of a 32.5 per cent acetic acid and allowed the mixture to stand for 13 months. Experiments had shown us that alcohol without the addition of acid will not separate the base from the dry "peptone." The acidulated alcohol was separated from the "peptone" by filtration, the alcohol driven off, the residue dissolved in a little water, sodium carbonate in excess was added, the mixture dried *in vacuo*, crushed to a powder, and extracted repeatedly with boiling chloroform. On evaporating the chloroform, a small amount of physiologically active basic material remained. This was dissolved in a very weak solution of sulphuric acid and freed from a little flocculent and fatty matter by filtration.

This solution caused a fall in blood pressure and stimulated the virgin uterus of the guinea-pig as shown in figures 13 and 14. The Pauly reaction was given with great intensity, the biuret reaction was negative, as one would expect since no proteose is soluble in chloroform. We regret not having weighed the residue as our experiment was thought at the time to be merely a preliminary one, to be followed by a more elaborate experiment later. Nevertheless when it is considered that we had in our hands a constituent of Witte's peptone, which is basic in character and which has the same action on blood pressure and plain muscle as the principle extracted from the hypophysis and other organs, and furthermore that the chloroform extract like that obtained from other tissues gave a positive Pauly reaction, we feel justified in assuming that the base in question is histamine. Now that we know that we are dealing with histamine, it will not be difficult to elaborate a method for the isolation of the base from Witte's peptone which is much superior to the imperfect extraction described above.

HISTAMINE, A HYDROLYTIC SPLIT-PRODUCT OF PURE PROTEINS

Believing as we do that histamine is one of the products of the enzymatic digestion of protein-containing foods, it was only natural that we should search for the amine among the decomposition products of pure proteins, when these are hydrolyzed by hydrochloric acid, alkaline solutions, or superheated steam. In the past, chemists have directed their attention almost exclusively to the isolation of amino-acids from among the hydrolytic split-products of proteins, not suspecting that small quantities of various physiologically active and perhaps highly significant amines (on the order of histamine) might also be present. It is our purpose to continue the investigations in this field which we have just begun and we content ourselves here with a brief summary of our findings in respect to the hydrolysis of pure albumin, casein and edestin with hydrochloric acid. The experiments are of a preliminary kind only, but the results are so thoroughly in agreement with those given in the preceding sections of our paper that one can have little doubt as to the existence of histamine among the split-products of pure proteins when hydrolyzed by any agents which are not secondarily destructive in their action. We find indications also of the existence of amines other than histamine among these split-products, but it remains for further investigation to furnish the complete proof of their presence.

PRELIMINARY EXPERIMENT WITH CRYSTALLIZED EGG ALBUMIN
CONTAINING A LITTLE GLOBULIN

Two grams of the albumin were dissolved as far as possible in a glass bowl in 20 cc. of concentrated hydrochloric acid, specific gravity 1.18 to 1.19, on the water bath. The acid was then immediately driven off on the bath under an electric fan, alcohol was repeatedly added and evaporated until but little acid remained. The residue was now extracted with absolute alcohol, the alcohol was driven off, the new residue taken up in water, filtered and treated with a 2 per cent solution of HgCl_2 in 0.8 per cent HCl . Mercury was removed from the filtrate in the usual way, the solution concentrated, made slightly alkaline with so-

dium hydroxide, filtered from a gummy precipitate, allowed to dry *in vacuo* over sulphuric acid and then exhausted repeatedly with absolute alcohol. On removing the alcohol of the extract, a slightly colored gummy precipitate remained. A small part of this lowered the blood pressure of a cat very markedly. As this residue still contained considerable albumose,⁷ it was again dissolved in water and five volumes of the above mentioned HgCl_2 solution were added. After filtration from a small precipitate,

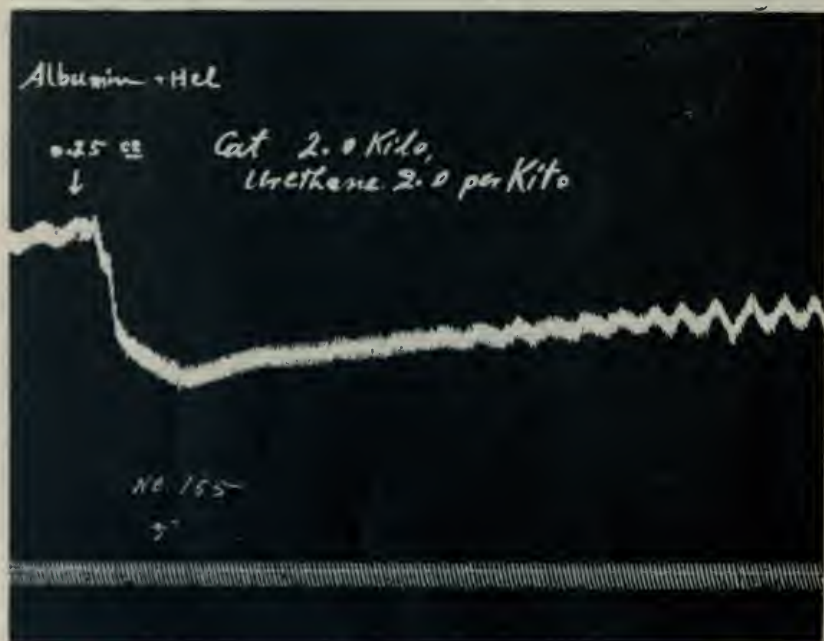


FIG. 15. SHOWS THE EFFECT OF A HYDROLYTIC PRODUCT OF ALBUMIN ON THE BLOOD PRESSURE OF THE CAT

the filtrate was freed from mercury, concentrated, freed from excess of acid taken up in water, neutralized, and tested on blood pressure (fig. 15). The outcome of this preliminary experiment led us to test other proteids in the same way as also to improve and to extend our methods.

⁷ We shall show in a forthcoming paper on albumoses and a-biuret peptids in the blood, that a certain amount of albumose or peptone, a little less than 1 mgm. per cubic centimeter, is easily removed in this way, the albumoses being completely adsorbed by the mercuric sulphide.

EXPERIMENTS WITH PURE CASEIN

To 5 grams of pure casein, prepared for the late Dr. A. H. Koelker some years ago by Kahlbaum, according to Hammarsten's directions, we added 100 cc. of recently distilled water and boiled for an hour under a reflux condenser, after which time the mixture was filtered, 10 cc. of the filtrate was concentrated to 1 cc. and this was injected intravenously into a cat weighing 2.1 kgm. The blood pressure fell from 110 mm. Hg to 82 mm. with the promptness characteristic of histamine. We calculate that the entire 100 cc. of aqueous extract obtained in the above experiment must have removed about 0.1 mgm. of histamine from the casein. It is not possible at present to decide whether this small quantity of the base was present as an impurity carried over from the milk, or whether it is a product of the hydrolytic action of the boiling water. In the latter case, we should have to regard the casein molecule as a labile one, in comparison with edestin, for example, which, when treated in the same way for two hours failed to part with even a trace of histamine.

Two grams of the pure casein were now hydrolyzed with concentrated hydrochloric acid on the water bath, and the products treated more or less according to the methods already described, when it was found that a depressor and uterine stimulating substance was here also readily obtainable. Thereupon, 50 grams of the casein were treated as follows: The 50 grams were dissolved in 500 cc. of concentrated hydrochloric acid, specific gravity 1.18 to 1.19, in small portions at a time with the assistance of the hot water bath, and the solution boiled gently for one hour on the bath. The hydrochlorid acid was then driven off, with the help of alcohol as before, the resulting, slightly acid product was extracted as far as possible with absolute alcohol, sodium hydroxide in alcohol was added to the filtered alcoholic extract until it became alkaline, the precipitate that fell out was separated, the filtrate acidulated with HCl and evaporated to a small volume, water was added and then much saturated solution of HgCl_2 , an abundant dark tarry precipitate was removed, and sodium hydroxide solution added to the filtrate. The flocculent mercury

compound which now fell out and which always contains histamine, if the base is present in a solution thus treated, was gathered at the centrifuge and washed three times with hot water, centrifuging each time. While washing in this way causes some loss of histamine, this is compensated for by the gains that follow the removal of a number of more soluble, physiologically inert,



FIG. 16. SHOWS THE EFFECT OF A CHLOROFORM EXTRACT OF THE HYDROLYTIC PRODUCTS OF CASEIN ON THE BLOOD PRESSURE OF THE CAT

mercury compounds. The washed precipitate was then decomposed in the usual manner, the filtrate from the mercuric sulphide was concentrated nearly to dryness and then dissolved in absolute alcohol. The alcohol was driven off, water was added and the product was again treated with HgCl₂ and NaOH as before. The product thus obtained was treated with Na₂CO₃,

dried *in vacuo* and extracted with chloroform. The residue remaining after evaporating the chloroform was found to be highly active physiologically and to behave in the manner characteristic of histamine as shown in figures 16 and 17.

The residue was alkaline to litmus, gave the Pauly reaction, a negative biuret test, and formed a crystalline picrate. According to estimations made on the blood pressure of the cat, 5 mgm. of the base were present in this residue and this amount we calcu-

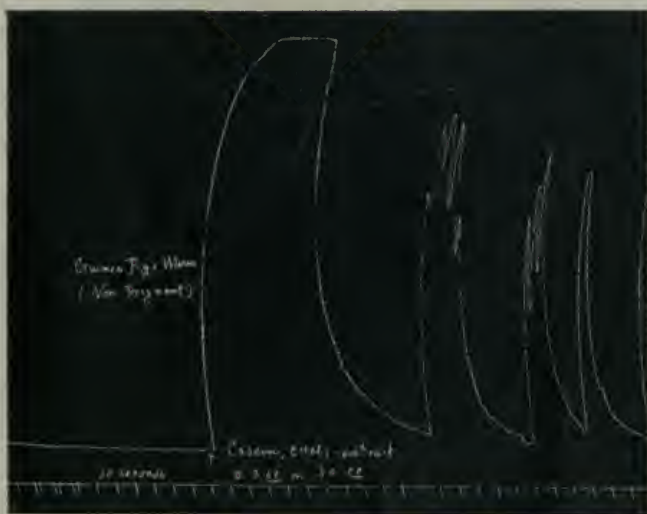


FIG. 17. SHOWS THE EFFECT OF A CHLOROFORM EXTRACT USED IN FIGURE 16 ON THE UTERUS OF THE VIRGIN GUINEA-PIG

A half horn was suspended in 30 cc. of Tyrode's solution. (Reduced to $\frac{2}{3}$).

late to be 15 per cent of that originally present in the hydrolyzed solution. No attempt was made to recover the base from bulky precipitates and incompletely extracted residues. Further purification of the chloroform extract was undertaken, but a description of the methods employed will be given in a later paper. From the results above obtained we can only conclude either that histamine itself, or an equally powerful homologue, which responds to the same tests, is one of the split-products of casein that has been hydrolyzed by hydrochloric acid.

EXPERIMENTS WITH CRYSTALLIZED EDESTIN⁸

A mixture of 100 cc. of water and 5 grams of crystallized edestin were boiled for two hours under a reflux condenser as already described for casein. At the end of this time the edestin was gathered into a rubber-like or fibrinous mass and the filtrate, when concentrated (10 cc. to 1 cc.) was found to be entirely devoid of action on the blood pressure of the cat. The boiled edestin was then decomposed with 50 cc. of concentrated hydrochloric acid as already described for casein. The subsequent treatment of the hydrolytic products was generally speaking like that described in the preceding section. The residue obtained on evaporating the chloroform was unusually large as compared with the similar residue from casein and albumin, but we are at present unable to say whether this is the result of certain changes in the methods preceding the extraction with chloroform or whether this proteid actually yields a larger amount of basic substances which are soluble in this reagent. As in the case of the other proteins, the basic residue was physiologically active (figs. 18 and 19).

We draw the same inferences here as we did in the case of the proteins, egg albumin, and casein and believe that nothing is required but further labor to isolate from edestin an absolutely pure crystalline compound with all the properties, chemical and physiological, that characterize histamine.

We may add that it would not surprise us if we should find that this or that member of the great family of proteins (perhaps even one of those here described) should yield not histamine itself, but one of its homologues, assuming for the moment that its homologues possess similar physiological properties.

GENERAL CONSIDERATIONS

We believe that we have demonstrated that the extremely active base, histamine, is a widely distributed constituent of all animal tissues, organ extracts in general, and enzymatic products,

⁸ Obtained from Prof. Lafayette Mendel who kindly sent us 20 grams of very pure edestin for use in our experiments.

such as Witte's peptone and erepton. Its presence among the hydrolytic decomposition products of the vegetable protein edestin, and its occurrence in the much used soya sauce of the Orient also point to the conclusion that the base is equally widely distributed among products derived from the vegetable kingdom.

Our experiments with freshly excised liver, excised muscle, edestin and other proteids, show that we take into the digestive

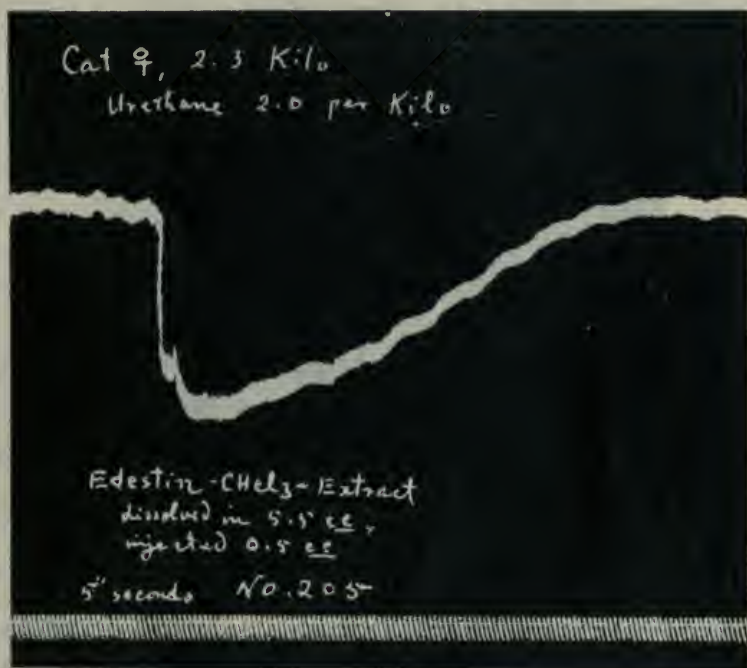


FIG. 18. SHOWS THE EFFECT OF A CHLOROFORM EXTRACT OF THE HYDROLYTIC PRODUCTS OF EDESTIN ON THE BLOOD PRESSURE OF THE CAT

tract a certain amount of the preformed base with every meal. Furthermore, our digestion experiments (HCl) with pure proteins would make it appear that an additional quantity of the base is produced during digestion itself. We are not in a position at present to give any very accurate data as to the actual amounts of the base that are daily taken by mouth. We calculate that 465 grams of boiled dog's liver contain 26.2 mgm. of the base.

To this must be added whatever is produced in the course of digestion, supposing this amount of liver to have been eaten. The 150 grams of erepton that were given in one meal to a large dog must have contained in the neighborhood of 225 mgm., as we judge from the depressor effect of an intravenous injection into a dog of 0.25 gram of the product. Liebig's meat extract, as we estimate by blood pressure measurements on the cat, contains nearly 0.5 mgm. of the base per gram of the extract.

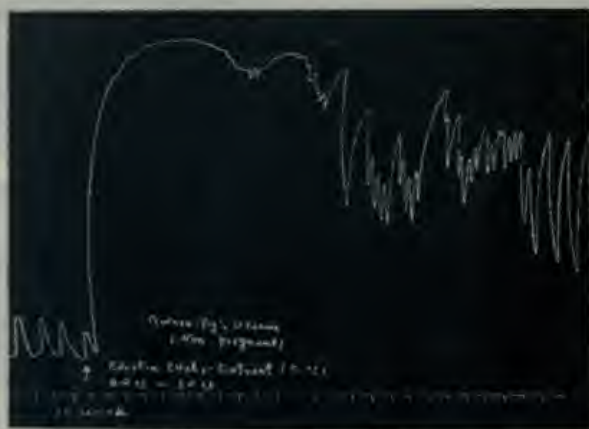


FIG. 19. SHOWS THE EFFECT OF THE CHLOROFORM EXTRACT USED IN FIGURE 18 ON THE UTERUS OF THE VIRGIN GUINEA-PIG

A half horn was suspended in 30 cc. of Tyrode's solution. (Reduced to $\frac{1}{2}$)

These few examples show that no inconsiderable quantities of this powerful plain muscle stimulant are consumed by us daily, and apparently, as we shall see, with advantage to the economy.

It is well known that an intravenous injection of a comparatively small amount of a given substance will destroy life while large quantities of it may be taken by mouth without the appearance, necessarily, of a single symptom. The various reasons for this are so familiar that they need not be given here.

In view of the fact that this potent substance is a constant constituent of our diet, one may well ask whether it does not normally play an important rôle as a stimulant for the gastric and intestinal musculature. That it is present in the digestive

canal in quantities which are adequate for this purpose cannot be denied. That the base itself as also its salts, and the peptones and albumoses of our diet (which always hold this base in chemical combination) can and do act as powerful stimulants for a strip of intestinal muscle has been shown by a number of writers. We ourselves had planned to introduce here a number of tracings, illustrating the action on the intestinal strip of histamine-containing food products, but later withdrew them as being superfluous. Medical experience, not to mention pharmacological experiments, with purgatives and numberless other drugs, proves conclusively that a soluble, absorbable agent, possessed of the stimulating properties of histamine, can not be taken by mouth without its pharmacological action on the musculature of the digestive organs becoming manifest. We conclude, therefore, that histamine plays a rôle, and perhaps a leading rôle, as a necessary stimulant for the musculature of the stomach and intestines. Anyone who has repeatedly injected into animals the ordinary secretin solutions of the laboratory, or albumose-peptone mixtures which contain histamine chemically bound to the albumoses, or any of the numerous organ extracts that contain the base, will have been so impressed with the increased peristaltic activity of the intestines immediately following these injections that he will not easily reject the conclusion here expressed.

In support of our opinion, we would cite the uses and physiological properties of soya-sauce. Those who are conversant with the life and customs of the people of Japan know how extensively this and other preparations of the soya bean are used in that country as accessory foods. Miso, a fermentation product of the bean, which is of the consistency of our peanut butter, when mixed with boiling water constitutes a breakfast dish, a sort of gruel, for almost all Japanese. The ordinary soya-sauce, which is a dark-colored liquid, is also used in the preparation of hot water mixtures which are consumed at the midday, but more often, at the evening meal. With Miso, vegetables may be incorporated for the breakfast dish, with the soya-sauce, vegetables and also fish. Dishes thus prepared are eaten by all

Japanese at least once and generally twice a day, and soya-sauce itself is also used at every meal as a condiment, in place of our salt, pepper, and sauces. It is a belief widely held by the Japanese people that these soya-preparations are necessary stimu-

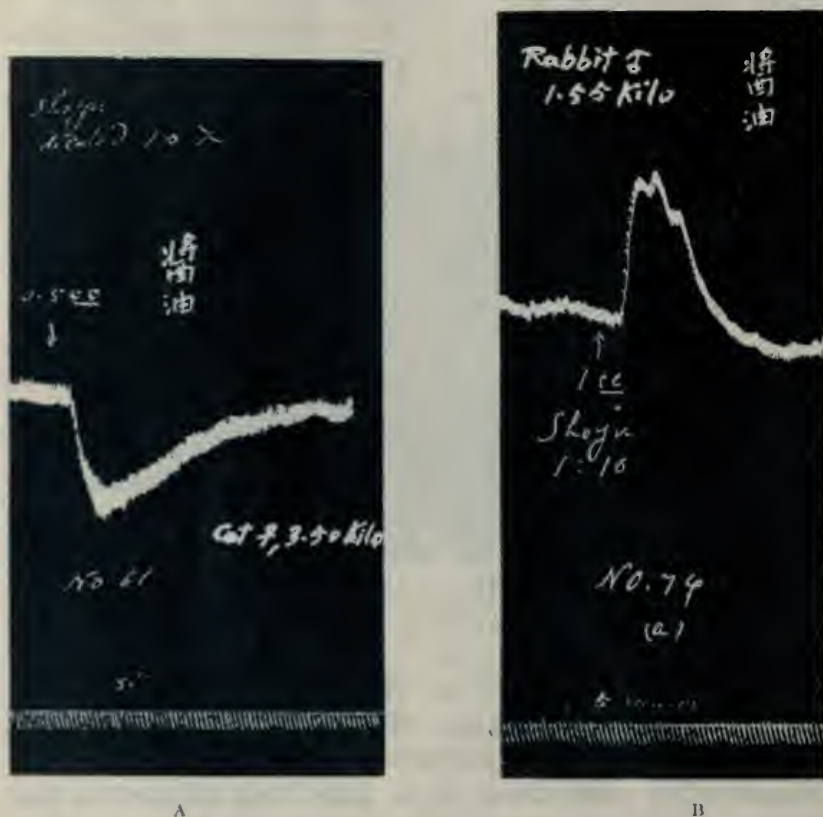


FIG. 20. SHOWS THE EFFECT OF SHOYU (JAPANESE SAUCE) ON THE BLOOD PRESSURE OF THE CAT (A) AND OF THE RABBIT (B)

Diluted 1:10, neutralized, and injected intravenously.

lants for the digestive apparatus, quite aside from their limited value as energy yielding foods. The quantity of these sauces daily ingested is small compared with the total amount of food consumed, but they nevertheless constitute an important element of the diet. Our own examination of an imported soya-sauce,

has made us certain that histamine is one of its constituents, the one in fact, which accounts for the stimulating action of the sauce on plain muscle. The action of the diluted sauce on the blood pressure of the cat and rabbit (see fig. 20, *A* and *B*), and on the uterus of the guinea pig are in entire agreement with that of histamine. We are aware that these soya-preparations may have other values for the economy than that of a gastro-intestinal stimulant, but in this respect, at least, these products of vegetable origin seem to serve the purpose of our meat extracts, meat soups and other stimulants derived from animal products.

The question arises here, whether any untoward effects follow the overconsumption of food products that contain considerable quantities of histamine. Dogs that receive a large quantity of erepton at one meal generally vomit soon after eating, and we have no doubt that the same result would follow an overdose of meat extract. Mellanby has found that vomiting and diarrhoea follow immediately upon the administration of the drug by mouth to a cat. Unfortunately, he fails to state the amount required to produce the effect. It remains for the future to study the remote or later effects of a diet containing a large amount of the base.

A young robust Eskimo may, during several months when seals are abundant, eat 4 kgm. of meat per day or even more. . . . Periods of abundant food are characterized by outbreaks of furunculosis, and it has been noticed further by medical officers and also by the natives, themselves, that in such periods many of them become extremely liable to profuse and frequent bleeding from the nose. (Quoted from A. and M. Krogh: *A Study of the Diet and Metabolism of Eskimos*, 1913, Copenhagen.)

The profuse and frequent bleedings from the nose may possibly be explained by the action of excessive quantities of histamine as brought out in the work of Dale and Richards, and Dale and Laidlaw.

Other inferences may perhaps be drawn from the action of the base upon the capillaries of the splanchnic area as demonstrated in the work of Dale and Richards and Dale and Laidlaw. We speak here of a degree of action on the capillary bed during diges-

tion which lies within normal limits and does not attain a degree of dilatation, and also of increased permeability of the capillaries which are evidently pathological in character, as described for toxic doses by Dale and Laidlaw. We raise the question, therefore, whether the histamine of our food does not play an important rôle during digestion as a relaxing agent for the capillaries of the gastric and intestinal mucosa. Such an action should greatly favor the passage of substances from the tissue spaces into the capillaries by a mere increase in the area of endothelial surface, not to speak of a possible alteration of the permeability of the endothelial cells. While this relaxing action of histamine on capillary areas should be most marked in the digestive tract during the hours of digestion, it is evident that this ability to widen capillary lumina may also be of great importance for other organs. Physiological theory has long postulated the need of a relaxing agent arising from cellular activity, to explain the increased rate of blood flow through an active organ, that is to say, one whose metabolism has become greatly accelerated. Thus, Barcroft and Piper (33) believe that the dilatation of the vessels of the submaxillary gland, caused by adrenalin, is due to metabolic products formed during the increased metabolism of the gland, rather than to the presence of dilator fibres in the sympathetic nerve supply of the gland. We, ourselves, as a result of the studies outlined in this paper, are inclined to accept the theory which holds that each organ of the body is so far "master of its own metabolism that it can force the vascular system to give it the oxygen" and the other products which it requires (33 a). Barcroft has shown the need for such an hypothesis in a convincing manner and he and his collaborators have made experiments, whose results support it rather than the alternative theory of stimulation via the nerve path. They have clearly shown that in the case of the submaxillary gland at least, we have a case of dilatation of which there is no satisfactory explanation other than that of a local chemical action. We shall not here comment further on this question, but would point out that the discovery of histamine in fresh tissues lends great support to the hypothesis. It only remains to show that this base is formed in increased

amount during functional activity, and this increase need be only minimal, in order to furnish the last link in the chain of evidence in favor of the theory. And that this point can be settled decisively in experiments in which chemical and physiological methods are combined, we do not doubt. Strong evidence in support of an increased production of histamine during functional activity is to be found in some of the otherwise inexplicable results of transfusion experiments on surviving organs, disconnected as these are from the excretory and other organs that would ordinarily eliminate or render innocuous an increased amount of the base. To be sure this evidence is weakened by the sound objection that an increased appearance of histamine in such transfusion experiments is not the result of increased metabolism of the normal kind, but arises rather from the death of numerous cells in an organ which is kept in an abnormal environment. The question must, therefore, be attacked with the help of new methods.

We believe that our results are also not without significance for a present day theory which asserts that a chemical factor predominates in the causation of traumatic shock. Some years ago, much experimental work was done both for and against the theory that extensive burns cause death in consequence of the absorption of toxic substances which appear at the site of the injury. And during the past few years military surgeons, physiologists, and pharmacologists seem independently to have reached the conclusion that shock, following upon extensive injury, especially of large masses of muscle tissue, is due to the absorption of a depressant substance from the injured tissue. Early in the war, the French surgeons, Quénu (34) and Delbet (35) arrived, independently of each other, at this conclusion from a study of surgical cases as also from experimental data. In the experimental field, however, the work of Dale and Laidlaw, and Dale and McIntosh, and others, on histamine shock and allied conditions as reported by Dale (36) early in 1917, laid a firm foundation for the theory of the chemical causation of the condition. The experiments of Bayliss (37) and Cannon (38) next demonstrated in a direct manner that a toxic, depressant substance is

absorbed from crushed tissue. These observers found that after severe mutilation of the thigh muscles, in decerebrate or anesthetized cats, the blood pressure falls only when the circulation through the blood vessels of the limb is reestablished. The fall also appears when the spinal cord has been divided and is, therefore, not of nervous origin. Massage of the injured vessels (the circulation of the limb being intact) results in a further fall of blood pressure with dilatation of the intestinal vessels. The chemical theory of shock seems, therefore, to be established.

We believe that our experiments demonstrate pretty conclusively that the depressant substance mainly concerned in traumatic shock is histamine. While the base was not isolated from dog's muscle and erepton as an absolutely pure salt, nevertheless, both the sulphate and the picrate which we had in hand exhibited the chemical and physiological properties which are characteristic of histamine and of no other known substance (see figs. 8, 9, 10, 11, and 12).

Since this toxic base always arises when tissues are subjected to trauma, and since the amount of the base liberated is probably proportional to the degree of injury and to the amount of tissue injured, it is evident that we have in this knowledge a rational basis for the wisdom of the age-long practice that inflamed or injured parts should be immobilized as far as possible.

Other consequences of both theoretical and practical significance follow the acceptance of this fact that histamine arises when tissues are injured. What form the agent that kills or injures the cell takes should be immaterial. Whether it be heat, mechanical energy, bacteria or their products, or pharmacological agents that injure or destroy the cell, in every instance we should expect to find histamine liberated. Nor need the injury necessarily be of so gross a character as to be immediately demonstrable under the microscope. Here evidently lie problems for future researches.

The reader will probably ask why we have not taken into consideration the possibility that more than one depressor substance is concerned in the phenomena that we have been discussing. During all of our experimental work, this question has been con-

stantly in our minds, and its solution, one of the aims of our research. We may state briefly that in all of our examinations of numerous tissue extracts, of Witte's peptone, of erepton, and of many precipitates and by-products appearing in the course of our manipulations, we have thus far not been able to extract any other depressant substance or plain muscle stimulant which in any degree approaches histamine in potency. Choline, for example, which may be encountered in small quantity in certain tissues, is one of the few substances that might be named in this connection, but its physiological, as also its toxic action, in general, is so feeble compared with that of histamine, that only a negligible part can be assigned to it in the production of the symptom complex that we have had under consideration. Methylguanidin, which occurs preformed in muscle would also appear to have only little action on the blood pressure, to judge from the experiments of Sinalnicoff and Bovshik (*Physiol. Abstracts*, ii, p. 358, 1917). Heyde (*Centralbl. f. Physiol.* xxv, p. 441, 1911) states that the fatal dose of the base for mice is 0.5 mgm., administered subcutaneously. Guinea pigs respond to an injection of 10 mgm. with symptoms similar to those seen after the administration of β -I., and this author concludes that methylguanidin probably plays a rôle in anaphylactic shock. The base used by him in his experiments was prepared from the urine of severely burned individuals and its purity may perhaps be questioned. While far less toxic than histamine, methylguanidin nevertheless deserves consideration in connection with the subjects discussed in this paper.

It may now be inquired whether the discovery of the occurrence of histamine in organ extracts, in Witte's peptone, etc., has any bearing on that important and intricate subject, anaphylaxis. Biedl and Kraus (39) first drew attention to the similarity of the symptoms of peptone shock to anaphylactic shock, and Dale and Laidlaw (40) then showed that the symptoms of peptone shock are again very largely identical with those of histamine, and "that the immediate symptoms with which an animal responds to an injection of a normally inert protein to which it has been sensitized, are to a large extent those of

poisoning by β -iminazolyl-ethylamine." We believe, for reasons stated in an earlier section of our paper, that the shock-producing constituent of Witte's peptone is in reality β -iminazolyl-ethylamine, which is chemically united with the albumoses of the mixture, but which is no less toxic in this than in any other soluble combination. We have not undertaken any work that bears immediately upon anaphylaxis, but would remark in leaving the subject for future consideration, that histamine is a cleavage product of proteins which is very easily liberated from the protein molecule, as witness the readiness with which it is produced by treatment of a proteid with hydrochloric acid of only a few minutes' duration. Secondly, while not wishing to commit ourselves here to any theory of anaphylaxis, we can see no reason for believing that a theory involving the production by ferment action of a toxic cleavage product, such as histamine or a substitution product of it, should encounter any opposition merely because of the time-relations involved. The suddenness of onset of the symptoms appears to many to preclude the possibility of the production of the poison by ferment action. One has only to assume that this action takes place over an extensive area, in innumerable cells or tissue spaces, and the difficulty vanishes. Many processes that go on in animal tissues are equally difficult to comprehend unless we bear in mind how extraordinarily the physical conditions existing in extensive surfaces, thin layers, etc., shorten the time factor, so that a quantity-effect or a quantity-production results in what would otherwise be an apparently impossible time.

In this connection also one calls to mind the important studies (41) of Vaughan on the poisonous group in the protein molecule and related subjects—studies extending over a period of years and begun before the phenomena of anaphylaxis were known. This investigator early found that a "poisonous group exists in widely diversified proteins, probably in all true proteins." He has also shown that the protein poison (which is not specific) can be obtained from the cellular substance of numerous saprophytic and pathogenic bacteria. The reader is referred to Vaughan's book for an account of his work and

that of his collaborators and for a statement of his views in regard to anaphylaxis, protein fever, specific ferments, and related subjects. Vaughan gives it as his opinion that the protein poison is the active principle in peptone, also "that it is the anaphylactic poison, and that it matters not what the agent be which has detached it from other groups" (42). For a long time there was no suggestion as to the chemical nature of the toxin of Witte's peptone and of Vaughan's protein poison. With the discovery of histamine and its physiological properties light began to be thrown on this question, as has been shown in the foregoing pages. Our own chemical and pharmacological studies have made us feel confident that the toxic principle of Vaughan's protein poison, as of the peptones and albumoses, is histamine. Apparently Vaughan, himself, has come to the same conclusion, for he writes, "With our poison, β -I. seems to agree closely. Both induce bronchial spasm and distention of the lungs in guinea-pigs, and cause prompt and marked fall in blood pressure in dogs. Neither destroys the coagulability of the blood. In the purest form in which we have obtained it our poison kills guinea-pigs intravenously in doses of 0.5 mgm., and this is the fatal dose of β -I. When the active agents in our crude poison are isolated we shall not be surprised if β -I. or some closely allied body is among them "(1913).

A word of comment on the pituitary principle as isolated by us may be permitted. We did not hastily arrive at the conclusion that the plain muscle stimulant of the pituitary gland is in reality histamine. It was only when both the chemical and the physiological evidence in favor of the identity of the two principles coincided at every point that we found no other conclusion tenable. As stated in the introduction to our paper, we have long considered it probable that the pituitary principle is not a hormone or substance specific to this organ, but is rather a widely distributed substance, everywhere the same, which may have its origin in the various tissues, in the gastric or intestinal mucosa, or which may be absorbed as such from among the products of digestion. Our supposition in regard to the wide distribution of the principle has been shown to have been well founded.

But many questions that were raised in our earlier paper still remain unanswered. For example, we have no adequate explanation for the relatively high concentration of histamine in the posterior lobe of the pituitary gland. Is more of the substance actually produced in this part of the gland, or are the local conditions such as to favor its accumulation here?

It will naturally be asked whether histamine is the only depressor substance and plain muscle stimulant of the gland, in other words, whether histamine is therapeutically equivalent to extracts of the gland, as a uterine and intestinal stimulant. We must give an affirmative answer to this question. Whenever, in our various chemical manipulations of extracts of the gland, a loss was met with, we took pains to recover the lost portion either in the form of a crude solution or of an impure salt. When the part thus recovered was tested on blood pressure and on the guinea-pig's uterus or intestinal strip, it was always found that the two physiological actions were in quantitative agreement, that is to say, where there was much blood pressure lowering substance, there also we found much of the uterine stimulant. Naturally we exclude from this consideration all substances except those that stimulate the uterus or intestine in minute doses only. No doubt one could bring forward other constituents of the pituitary gland or of other organs of the body which are feeble stimulants of plain muscle or feeble depressants of blood pressure, but to do this, would not invalidate our conclusion that histamine is the essential, therapeutically active constituent of the hypophysis.

The pressor constituent of the gland has not yet been isolated and we are entirely ignorant in regard to its chemical nature. Its presence in pituitary extracts is no doubt of some therapeutic importance as it probably serves in the small doses, in which these extracts are employed in medical practice, as a counteracting agent to the depressor effect of histamine on the circulation. The two principles, histamine and the still unknown blood pressure raising constituent, mixed in certain favorable proportions, as they appear to be in extracts of the gland, give us a very serviceable pharmacopoeial preparation

so to speak. It should not be beyond the skill of pharmacologists to replace this unknown pressor substance by a known principle, as say, parahydroxyphenylethylamine which is present in ergot, and thus produce a pharmacopoeial⁹ preparation equally serviceable with the present high priced pituitary extracts. When the pressor principle, itself, shall have been isolated a more exact imitation of nature's alchemy will be possible.

We have shown that we daily consume in our food no inconsiderable amounts of histamine, and we have also called to mind the fact that a substance, which is highly poisonous when given intravenously or when it is rapidly absorbed from the tissue spaces, may be quite innocuous or even beneficial when taken in much larger quantities by mouth especially at meal time. We must, therefore, inquire as to the quantity of histamine that can be tolerated, its fate in the body, and its mode of excretion. Our knowledge in regard to these points is only fragmentary at present.

Dale and Laidlaw (43) injected 10 mgm. of the hydrochloride into the long saphenous vein of a cat and observed that immediate vomiting and purging, profuse salivation and labored respiration, with a subsequent period of collapse and light narcosis, resulted. At the end of an hour when the cat had partially recovered, a second intravenous injection of 20 mgm. was made. Vomiting and collapse again occurred, but the symptoms were rather less marked than after the first injection of 10 mgm. The animal recovered completely during the night.

The effects of the subcutaneous injection of quantities of the hydrochloride as large as 50 mgm. or even 100 mgm., were naturally somewhat slower in onset, but otherwise similar to those described. The cats thus treated also recovered completely.

Oehme (44) states that the fatal dose for rabbits after intra-

⁹ At the moment of going to press we find that this suggestion has already been acted upon. A. Hoerlein, U. S. Patent, 1,178,720, April 11, 1916 (see Abstracts, Amer. Chem. Soc. 10³, 1577, 1916), combines para-hydroxyphenylethylamine and histamine in the proportions of 1-4 to 30 parts in aqueous solution, as a substitute for preparations of ergot, and states that the mixture is free from disagreeable after effects.

venous injection is 0.6 mgm., or even less, of the hydrochloride per kilogram of body weight, but when the infusion into the vein was made very slowly, a rabbit weighing 2.175 kgm. tolerated 12.9 mgm. without showing any symptoms, and a second rabbit weighing 2.6 kgm. tolerated 15.7 mgm. of the salt injected in the same way. Guinea-pigs, as is well known, are also very sensitive to the base. Intravenous injections of 0.5 mgm. into large guinea-pigs weighing 800 to 1060 grams cause death in a few minutes.

In regard to the fate of the base in the organism, we are but ill informed. Dale and Laidlaw obtained some evidence of a slight disappearance of the substance in the liver during perfusion experiments with this organ and state that the limit of the destructive power of the liver is soon reached. They state that histamine is not excreted as such in the urine. The urine of a cat that had received 150 mgm. in all (subcutaneously) was collected during the forty-eight hours succeeding the first injection and was found to have no perceptible effect on the isolated uterus of the guinea-pigs in doses of 5 cc. As this urine gave the Pauly reaction with great intensity, it was inferred that histamine is excreted in the form of a compound devoid of the characteristic physiological activity but with the iminazole ring intact.

Oehme, however, found in two out of three experiments with rabbits that the urine of these animals contained the base in an active form in a concentration of $1:5 \times 10^6$ and expressed the opinion that this very limited excretion can not play any part in reducing the toxicity of histamine.

Mellanby (45) concludes from his experiments that histamine is not absorbed from the large intestine, but that its absorption in the small intestine increases from the duodeno-jejunal flexure to the caecum, that is, the nearer the caecum, the greater is the absorptive capacity of the small intestine.

What are the chemical changes that take place in that fraction of histamine which is metabolized and rendered innocuous in the body? This question can at present be answered only by assuming that its disposal in the body is analogous to that of

certain other amines (46), and Mellanby accordingly suggests that the base after absorption into the portal circulation is deaminated by the liver cells, the alcohol side chain $-\text{CH}_2\text{CH}_2\text{OH}$, which has now taken the place of the grouping $-\text{CH}_2\text{CH}_2\text{NH}_2$, is then oxidized to $-\text{CH}_2\text{COOH}$, giving an acetic acid compound which in turn may unite with glycine and be excreted as the aceturic compound.

In conclusion it may be stated that it remains for future investigation to determine the form of union in which histamine is held in the protein molecule. It has been shown that digestion with hydrochloric acid for a short time liberates histamine from proteins, and it is natural to conclude that the histidin group of the protein is the immediate precursor of the base. When pure histidine was subjected to the same treatment on the water bath with hydrochloric acid, as was given to edestin and other proteins in the experiments described in this paper, it yielded not even a trace of histamine. This is what one would expect, as the decarboxylation of an amino-acid can not ordinarily be effected by such mild treatment. This point will receive further attention in a later paper.

SUMMARY

Our main conclusions may be stated as follows:

1. Histamine, β -iminazoly-ethylamine, a substance which stimulates plain muscle tissue in minute doses, which depresses the circulation, and which causes a shock-like prostration when administered in doses that lie beyond the limits of toleration, is a widely distributed constituent of all animal tissues, organ extracts, and enzymatic products, such as Witte's peptone and erepton, whether derived from animal or vegetable proteids. While certain bacteria, as is well known, are capable of producing the base by decarboxylating histamine, its occurrence as here described is entirely independent of micro-organisms. Hydrolysis of pure proteins, such as crystallized albumin, pure casein, and edestin, with hydrochloric acid yields a base which is identical in our opinion with histamine or which at best is a similarly acting substitution product of it.

2. It follows from the above that histamine is a constituent of our diet and that we daily consume no inconsiderable amount of the base, some of which is present in the form of the free base or a simple salt, while more of it is in all probability produced in the course of digestion. We base the latter assertion on the fact that enzymatic products, such as Witte's peptone and erepton, contain the base in larger amount than the original material from which these products are derived.

3. We have suggested that histamine plays an important rôle as stimulant for the gastric and intestinal musculature and also as a dilator of capillaries during digestion. This last action is probably also of importance for organs in general, during periods of increased activity. It has also been suggested that histamine is the most powerfully acting among the depressant substances which have their origin in mutilated tissues, and hence plays the leading rôle among the chemical factors concerned in traumatic shock.

4. Histamine is the plain muscle-stimulating and depressor constituent of the posterior lobe of the pituitary gland. The physiological and chemical evidence in favor of the identity of the two principles coincide at every point. As histamine occurs to some extent in all tissues, it can no longer be considered to be a hormone or substance specific to the pituitary gland. We have no explanation to offer at present for the relatively high concentration in which the base is found in the posterior lobe of this organ.

5. We have given a few data to prove that the animal organism can tolerate considerable amounts of histamine when it is given by mouth and have gathered from the writings of other investigators the facts now known and the surmises in regard to the excretion of the base and its fate in the organism.

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A COMPARISON OF THE INFLUENCE OF SECRETINE AND THE ANTINEURITIC VITAMINE ON PANCRE- ATIC SECRETION AND BILE FLOW

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INTRODUCTION

In the course of an investigation having for its purpose the elucidation of the physiological function of vitamins, it occurred to us that the antineuritic vitamin (or water soluble B) might be concerned in the stimulation of the digestive glands. This hypothesis was based on some previous observations on the sequence of events following the treatment of polyneuritic pigeons by means of an active antineuritic vitamin preparation. When a preparation of this type is given to a paralyzed bird it is found that after about one to two hours the animal voids a large amount of intensely bile stained material. As a rule, this is followed by a marked improvement in the paralytic symptoms. It was reasonable to interpret this observation either (1) by assuming that the vitamin stimulates peristalsis, resulting in the discharge of accumulated bile or (2) that the vitamin actually increases the flow of bile.

The latter explanation had much in its favor for the reason that secretine and antineuritic vitamin seems to possess very similar, if not identical chemical and physical properties, as will be shown later on. Hence it was important to decide the following questions:

1. Is an intravenous injection of an active vitamin preparation followed by an increase of pancreatic and bile secretion?
2. Does the upper intestine contain a substance with curative properties for polyneuritic pigeons?

3. It is possible to purify both secretine and antineuritic vitamine by the same procedures.

The data obtained in this work is compiled in the following section of this paper.

EXPERIMENTAL PART

For the preparation of the secretine solutions a modification of the method of Bayliss and Starling (1) was employed, using the upper part of the small intestine of hogs. The hogs had been starved previous to slaughtering for from twenty-four to forty-eight hours. The intestines were opened, thoroughly washed with cold water and the water removed as far as practicable. The mucosa was then scraped off and the pulp weighed. The latter was ground thoroughly with washed quartz sand and extracted with 95 per cent methyl alcohol (containing 1 cc. concentrated HCl per liter) in the proportion of 2 cc. of alcohol per gram of pulp. This mixture was heated on the water bath until the alcohol began to condense on the lower portions of the condenser. This temperature was maintained for three hours. After filtering from the solid residue, the filtrate was evaporated in vacuum at a low temperature. The lipoid material contained in the residue was removed by ether and the ether layer shaken out with a little slightly acidulated water and the aqueous portion made up to a definite volume. This solution was called crude *secretine* (*S2*).

One cubic centimeter of secretine 2 equals 12.5 grams pulp.

Secretine 4. (S4). *S2* was treated with an excess of a hot saturated solution of silver acetate, which precipitated the chlorides and purines. This precipitate was filtered off with suction and the filtrate treated with a hot saturated solution of barium hydroxide until the solution became just slightly alkaline to litmus. The precipitate formed was filtered off rapidly with suction, washed with cold distilled water and suspended in water containing a small amount of sulphuric acid.¹ During decomposition

¹ It is essential to add enough sulphuric to convert all of the barium into the sulphate in order to prevent the passage of this highly toxic substance into the final preparation.

with hydrogen sulphide the lumps were well broken up. After all of the silver had been converted into sulphide the latter was filtered off and the excess of hydrogen sulphide removed in vacuum. The sulphuric acid was then removed by treatment with a slight excess of lead acetate and after the removal of the lead sulphate the excess of lead was removed with hydrogen sulphide. The clear filtrate from the lead sulphide was concentrated in vacuum at about 30 to 40°C.

One cubic centimeter of this solution (S4) corresponds to 10 grams of original pulp.

This solution yielded a heavy precipitate with phosphotungstic acid and assumed upon the addition of a saturated solution of sodium carbonate a deep blue color. It gives a positive diazo test with diazotized sulphanilic acid. The biuret is negative and there is formed no immediate precipitate upon the addition of picric acid, although a turbidity appears on standing. The solution gives a positive ninhydrin reaction. An alkaline solution of pure histidine also yields a positive ninhydrin reaction.

Secretine 5. (S5) was obtained in exactly the same way as S4 using another batch of hog's intestines.

One cubic centimeter of S5 equals 13 grams of pulp.

Thirty-seven cubic centimeters of S5 were treated with mercuric sulphate reagent prepared according to the directions of Kossel and Patten (2). The precipitate was filtered off and after suspending it in water, decomposed with hydrogen sulphide. The excess of sulphuric acid was removed by means of lead acetate, filtered and the excess of lead removed by hydrogen sulphide. The filtrate from the lead sulphide was concentrated, so that 1 cc. did correspond to 26 grams of pulp. The solution was called *secretine 6* (S6) and represents the histidine fraction.

The filtrate previously obtained upon the addition of mercuric sulphate to S5 was treated with absolute ethylalcohol until precipitation was complete. The precipitate was filtered off, suspended in water containing a small amount of sulphuric acid and decomposed with hydrogen sulphide. The sulphuric acid in the filtrate from the HgS was removed by means of lead acetate.

The filtrate was freed from lead by means of hydrogen sulphide. The filtrate from the lead sulphide was concentrated in vacuum at low temperature.

One cubic centimeter of this solution *secretine 7* (S7), equals 19 grams of pulp.

The filtrate from the alcohol insoluble fraction was carried down in vacuum to remove the alcohol. It was then taken up in water and a little sulphuric acid and subjected to the same procedure as *secretine 7* for the removal of mercury, sulphuric and lead. This preparation is called *secretine 8* (S8) of which 1 cc. corresponds to 19 grams of pulp.

Yeast vitamine 133, 134 and 138. For each lot of these preparations 800 grams of finely ground dried brewers yeast were extracted twice on the water bath with 1600 cc. of methyl alcohol (containing 1 cc. concentrated HCl per liter). The alcohol was removed in vacuum and the residue acidulated with HCl and treated with water and ether in order to remove the lipid material. The aqueous extract was concentrated in vacuum to about 1 liter. The concentrate was subjected to the silver barium and lead treatment as described for *secretine 4*.

One cubic centimeter of yeast vitamine 133 corresponds to 20 grams of dried yeast.

One cubic centimeter of yeast vitamine 134 corresponds to 13 grams of dried yeast.

One cubic centimeter of yeast vitamine 138 corresponds to 10 grams of dried yeast.

Properties of yeast preparations. These solutions yield a heavy precipitate with phosphotungstic acid which gives a blue solution upon the addition of an excess of sodium carbonate. The biuret is negative; ninhydrine positive; diazotized sulphianilic test positive and picric acid forms a precipitate on long standing.

Normal cat secretine. The intestinal pulp was obtained in the same manner as described for *secretine 2*, except that ethyl alcohol was used in place of the methyl alcohol.

One cubic centimeter of the final solution corresponds to 1 gram of intestinal pulp.

*Polyneuritic cat 101 secretine.*² This secretine solution was prepared in the same way as that of the normal cat.

One cubic centimeter of the final solution corresponds to 0.5 gram of intestinal mucosa.

Curative properties of preparations as tested on polyneuritic pigeons

Secretine 2. A pigeon which showed marked leg and wing paralysis was given 8 cc. of secretine 2 (corresponds to 100 grams of intestinal pulp) per os at 10.00 a.m. At 4.00 p.m. on the same day the bird walked very well showing only slight paralysis, but it was unable to fly. Two days later the pigeon showed no symptoms whatsoever. Three days later the bird had a severe relapse. Another dose of 10 cc. secretine 2 brought about marked temporary improvement.

Another pigeon with marked leg paralysis was given 10 cc. of secretine 2 per os and was greatly improved with 20 hours. Another dose of 10 cc. of this preparation brought about the complete disappearance of all symptoms. Another relapse on the sixth day was greatly improved by another dose of 10 cc.

A third pigeon with severe leg and wing paralysis received 10 cc. of secretine 2. Seventeen hours later the bird flew normally and the leg paralysis was greatly improved, so that the bird could walk about. The improvement persisted for several days.

Secretine 4. A polyneuritic pigeon which was still well nourished and showing a staggering gait, falling over frequently, received 5 cc. secretine 4 (corresponding to 50 grams of fresh intestinal pulp) per os. The following day the bird was improved. Another dose of 10 cc. of secretine 4 lead to the complete disappearance of the paralytic symptoms.

Another polyneuritic bird whose symptoms had been relieved by the administration of a yeast preparation developed a relapse twelve days later and was then greatly improved by the oral administration of 10 cc. of secretine 4. It was able to run

² Cat 101 was used in another investigation (3) and had been fed with beef deficient in antineuritic vitamine. As a result of this dietary deficiency the animal developed severe symptoms of polyneuritis and subsequently died. The upper intestine of this cat was worked up soon after death.



FIG. 1. EXPERIMENT 1. DOG, 20 KILO

Note marked stimulating effect on pancreatic and biliary secretion of an injection of 5 cc. yeast vitamin 133 (equal to 100 grams of yeast). This preparation corresponds to Funk's vitamin fraction (pyrimidine fraction).



FIG. 2. EXPERIMENT 2. DOG, 8 KILO

Shows stimulating effect of yeast vitamin 134 (5 cc. represent 65 grams of yeast) on pancreatic and biliary secretion. This preparation corresponds to Funk's vitamin fraction.

fairly well, whereas it had shown severe leg paralysis previous to the administration of the secretine.

Yeast vitamine 133. A polyneuritic pigeon which walked with a staggering gait and often fell over, was given 1 cc. of this preparation (corresponding to 20 grams of dried yeast). Four hours later the bird was able to fly and run normally. The improvement persisted 14 days when the bird was changed to a mixed diet.

Yeast vitamine 134. A well nourished polyneuritic pigeon which was unable to walk was given 2 cc. of this preparation (corresponding to 26 grams of dried yeast). Seventeen hours later the bird was found greatly improved, being able to fly and run normally. The symptoms did not reappear for eight days, when the bird was changed from rice to a mixed diet.

Experiment 1. Dog, 20 kilo; light chloretone anaesthesia. Blood pressure measure in carotid by means of a mercury manometer, cannula inserted into pancreatic duct. A second cannula was inserted into common bile duct. Injections made into femoral vein.

2.01–2.11 p.m. 2 drops pancreatic secretion; no bile.

2.11 p.m. Injection of 5 cc. *yeast vitamine 133* (equal to 100 grams yeast), followed by 42 mm. fall in blood pressure (see fig. 1).

2.11–2.21 p.m. 18 drops pancreatic secretion; 16 drops of bile.

2.21–2.31 p.m. 6 drops pancreatic secretion; 7 drops of bile.

3.24–3.34 p.m. 3 drops pancreatic secretion; 2 drops of bile.

3.34 p.m. Injection of 3 cc. *yeast vitamine 133* (equal to 60 grams yeast), followed by 44 mm. fall in blood pressure.

3.34–3.44 p.m. 7 drops pancreatic secretion; no bile.

3.44–3.54 p.m. 3 drops pancreatic secretion; no bile.

3.54–4.02 p.m. 2 drops pancreatic secretion; 1 drop of bile.

4.02 p.m. Injection of 1 cc. *secretine 2* (equal to 12.5 intestinal pulp), followed by 62 mm. fall in blood pressure (see fig. 4).

4.02–4.12 p.m. 296 drops pancreatic secretion; 42 drops of bile.

4.12–4.22 p.m. 157 drops pancreatic secretion; 41 drops of bile.

4.22–4.32 p.m. 18 drops pancreatic secretion; 9 drops of bile.

4.32–4.42 p.m. 5 drops pancreatic secretion; 8 drops of bile.

Experiment 2. Dog, 8 kilo; deep chloretone anaesthesia.

11.07–11.17 a.m. 0 drops pancreatic secretion; 0 drop of bile.

11.17 a.m. Injection of 0.75 cc. *secretine 4* (equal to 7.5 grams of pulp), followed by 34 mm. fall in blood pressure (see fig. 5).



FIG. 3. EXPERIMENT 3. Dog, 18 Kilo

Shows that a yeast preparation (5 cc. yeast vitamine, equal to 50 grams of yeast) obtained in exactly the same way as yeast vitamine 133 (see figure) has no effect on pancreatic and biliary secretion. This evidently shows that this preparation has lost its action on the pancreas. It also has lost its curative properties for polynouritic pigeons, but it still has retained its marked depressor effect, proving that depressor action and action on pancreatic secretion are independent of each other, which also applies to secretine.



FIG. 4. EXPERIMENT 1. Dog, 20 Kilo

Note marked stimulating effect on pancreatic secretion and bile flow of an injection of 1 cc. secretine 2, corresponding to 12 grams of intestinal pulp. This preparation is a crude, fat free extract of intestinal pulp from hogs.

- 11.17-11.27 p.m. 15 drops pancreatic secretion; 4 drops of bile.
12.23-12.33 p.m. 0 drops pancreatic secretion; 2 drops of bile.
12.33 p.m. Injection of 6.5 cc. *polyneuritic cat 101 secretine* (equal to 3.2 grams pulp), followed by no change in blood pressure.
12.33-12.43 p.m. 0 drops of pancreatic secretion; 3 drops of bile.
1.23-1.33 p.m. 0 drops of pancreatic secretion; 0 drops of bile.
1.33 p.m. Injection of 1 cc. *secretine 4* (equal to 10 grams of pulp), followed by 34 mm. fall in blood pressure.
1.33-1.43 p.m. 27 drops pancreatic secretion; 5 drops of bile.
1.52-2.02 p.m. 0 drops pancreatic secretion; 0 drops of bile.
2.02 p.m. Injection of 5 cc. *yeast vitamine 134* (equal to 65 grams dried yeast), followed by 20 mm. fall in blood pressure (see fig. 2).
2.02-2.12 p.m. 7 drops of pancreatic secretion; 5 drops of bile.
2.18-2.28 p.m. 0 drops of pancreatic secretion; 7 drops of bile.
2.28 p.m. Injection of 5 cc. *secretine 4* (equal to 50 grams pulp).
2.28-2.38 p.m. 23 drops of pancreatic secretion; 7 drops of bile.
Experiment 3. Dog, 18 kilos; light chloretone anaesthesia.
10.55-11.05 a.m. 0 drops of pancreatic secretion.
11.05 a.m. Injection of 2 cc. *secretine 5* (equal to 26 grams pulp), followed by 60 mm. fall in blood pressure.
11.05-11.15 a.m. 47 drops pancreatic secretion.
11.16-12.26 a.m. 3 drops pancreatic secretion.
11.26 a.m. Injection of 5 cc. *secretine from normal cat* (equal to 5 grams intestinal pulp), followed by 20 mm. fall in blood pressure.
11.26-11.36 a.m. 28 drops of pancreatic secretion.
11.36-11.46 a.m. 2 drops of pancreatic secretion.
11.46 a.m. Injection of 1 cc. *yeast vitamine 138* (equal to 10 grams of dried yeast), followed by 52 mm. fall in blood pressure and no flow of pancreatic secretion (see fig. 3).
12.24 p.m. Injection of 5 cc. *yeast vitamine 138* (equal to 50 grams dried yeast), followed by 60 mm. fall in blood pressure and no flow of pancreatic secretion.
12.41-12.51 p.m. 1 drop of pancreatic secretion.
12.51 p.m. Injection of 2 cc. *secretine 5* (equal to 26 grams of pulp), followed by 48 mm. fall in blood pressure.
12.51-1.01 p.m. 26 drops of pancreatic secretion.
1.51-2.01 p.m. 2 drops of pancreatic secretion.
2.01 p.m. Injection of 3 cc. *secretine 5* (equal to 39 grams of pulp), followed by 50 mm. fall in blood pressure.
2.01-2.11 p.m. 53 drops of pancreatic secretion.



FIG. 5. EXPERIMENT 2. Dog, 8 Kilo

Shows the stimulating effect of a small amount of secretine 4 (0.75 cc. equal to 7.5 gram intestinal pulp) on pancreatic and biliary secretion. This preparation represents the pyrimidine fraction and corresponds to Funk's vitaminic fraction.



FIG. 6. EXPERIMENT 4. Dog, 20 Kilo

Shows stimulating effect of an injection of 5 cc. secretine 6 (equal to 130 grams intestinal pulp) on pancreatic and biliary secretion. This preparation represents the insoluble fraction obtained by means of mercuric sulphate and corresponds to the histidine fraction.

- 2.11–2.15 p.m. 2 drops of pancreatic secretion.
2.15 p.m. Injection of 4 cc. *secretine* 4 (equal to 40 grams of pulp),
followed by 32 mm. fall in blood pressure.
2.15–2.25 p.m. 43 drops of pancreatic secretion.
2.27–2.32 p.m. 1 drop of pancreatic secretion.
2.32 p.m. Injection of 5 cc. *normal cat secretine* (equal to 5 grams pulp)
followed by 18 mm. fall in blood pressure.
2.32–2.37 p.m. 7 drops of pancreatic secretion.
Experiment 4. Dog, 20 kilos; ether, anaesthesia. Animal had been
fed five hours previously and was showing active digestion, which may
explain the marked bile flow previous to the injections.
10.49–10.59 a.m. 3 drops of pancreatic secretion; 81 drops of bile.
10.59 a.m. Injection of 1.5 cc. *secretine* 7 (equal to 29.5 grams of pulp),
followed by 44 mm. fall in blood pressure.
10.59–11.09 a.m. 9 drops of pancreatic secretion; 77 drops of bile.
11.25–11.35 a.m. 2 drops of pancreatic secretion; 34 drops of bile.
11.35 a.m. Injection of 2.2 cc. *secretine* 5 (equal to 28.6 grams of pulp),
followed by 80 mm. fall in blood pressure.
11.35–11.45 a.m. 40 drops of pancreatic secretion; 0 drops of bile.
11.50–12.00 a.m. 2 drops of pancreatic secretion; 0 drops of bile.
12.00 m. Injection of 3 cc. *secretine* 7 (equal to 66 grams of pulp), fol-
lowed by 62 mm. fall in blood pressure (see fig. 7).
16 drops of pancreatic secretion; 54 drops of bile.
1 drop of pancreatic secretion; 37 drops of bile.
1.36–1.46 p.m. 0 drops of pancreatic secretion; 13 drops of bile.
1.46 p.m. Injection of 5 cc. *secretine* 6 (equal to 130 grams of pulp),
followed by 58 mm. fall in blood pressure (see fig. 6).
1.46–1.56 p.m. 12 drops of pancreatic secretion; 37 drops of bile.
2.10–2.20 p.m. 0 drops of pancreatic secretion; 13 drops of bile.
2.20 p.m. Injection of 5 cc. *secretine* 8 (equal to 95 grams of pulp),
followed by 16 mm. fall in blood pressure (see fig. 8).
2.20–2.30 p.m. 0 drops of pancreatic secretion; 14 drops of bile.
2.35–2.45 p.m. 0 drops of pancreatic secretion; 19 drops of bile.
2.45 p.m. Injection of 5 cc. *yeast vitamine 138* (equal to 50 grams dried
yeast), followed by 90 mm. fall in blood pressure.
2.45–2.55 p.m. 1 drop of pancreatic secretion; 32 drops of bile.
3.00–3.05 p.m. 0 drop of pancreatic secretion; 16 drops of bile.
3.05 p.m. Injection of 4 cc. *secretine* 7 (equal to 76 grams of pulp), fol-
lowed by 68 mm. fall in blood pressure.
3.05–3.15 p.m. 21 drops of pancreatic secretion; 49 drops of bile.



FIG. 7. EXPERIMENT 4. Dog, 20 Kilo

Note marked stimulating effect on pancreatic and biliary secretion of an injection of 3 cc. secretin 7 (equal to 66 grams of intestinal pulp). This preparation was obtained by adding alcohol to the filtrate from secretin 6, causing a precipitate to form which was decomposed by hydrogen sulphide. The filtrate from the precipitate was worked up and constitutes secretin 8 (see chart 8).



FIG. 8. EXPERIMENT 4, Dog, 20 Kilo

Shows that secretine 8 is inactive as far as its influence on the pancreatic and biliary secretion is concerned. Note the insignificant effect on blood pressure which is in marked contrast to some of the active fractions.

3.35-3.45 p.m. 0 drops of pancreatic secretion; 18 drops of bile.

3.45 p.m. Injection of 5 cc. *secretine 6* (equal to 130 grams of pulp), followed by 72 mm. fall in blood pressure.

3.34-3.55 p.m. 29 drops of pancreatic secretion; 48 drops of bile.

3.55-4.05 p.m. 0 drops of pancreatic secretion; 29 drops of bile.

DISCUSSION OF RESULTS

From the results obtained in this research it is evident that a purified yeast extract when injected intravenously into dogs, stimulates pancreatic secretion and bile flow. It furthermore appears that a yeast vitamine preparation (yeast vitamine 138) which has lost its curative properties for avian polyneuritis is devoid of any stimulating influence on pancreatic secretion and bile flow. Such an inactive preparation still possesses its depressor action on blood pressure, proving that the depressor substance of the yeast extract is not identical with the substance causing the increase in pancreatic secretion. In this connection it should be pointed out that the same difference between depressor and secretory substance also obtains in the case of secretine preparations derived from the duodenum, as was shown by Launoy and Oechslin (4). These authors were able to prepare a fraction which produced only feeble pancreatic secretion, but caused a very marked fall in blood pressure.

The work on the chemical purification of both secretine and antineuritic vitamine has shown that both principles are found in the same fractions and some unpublished work of Voegtlin and George F. White has demonstrated that the behavior of secretine and antineuritic vitamine towards Lloyd's reagent (hydrous aluminium silicate) is identical, inasmuch as both substances are removed from their solutions by treatment with this reagent. Both substances can be recovered from the fuller's earth by suitable treatment with alkali.

After studying the protocols of these experiments it will become obvious that purification of the secretine by various chemical means leads to a gradual loss in physiological activity. A similar loss in physiological activity was experienced in our work on the chemical isolation of vitamine.

We have also shown that the wall of the duodenum of animals, who had not received any food for at least twenty-four hours, contains both secretine and antineuritic vitamine in fairly large amounts.

It is rather significant that an extract of the duodenum of a polyneuritic cat had no influence on pancreatic secretion, whereas an extract of a normal cat's duodenum caused a marked increase in pancreatic flow. This last observation is based only on one experiment and for this reason is hardly conclusive. The work is now being repeated on a larger scale.

The observations reported in this paper would suggest that the antineuritic vitamine and secretine are possibly one and the same substance. Additional proof for this hypothesis and a discussion of its significance will be presented in a subsequent communication.

CONCLUSIONS

1. By employing proper methods it is possible to obtain an extract from brewers yeast which causes an increase in pancreatic secretion and bile flow, when this extract is injected intravenously into dogs.

2. Secretine solutions obtained from the duodenum of hogs by the methods described in this paper evidently contain a considerable amount of antineuritic vitamine, as shown by their power in relieving the paralytic symptoms of polyneuritic pigeons.

3. The chemical and physical properties of secretine and vitamine are similar if not identical. Both substances are found in the same fractions.

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EXPERIMENTAL IRRIGATION OF THE SUBARACHNOID SPACE

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The extremely high mortality attending pyogenic infections of the meninges with organisms other than the diplococcus intracellularis and the prospects of a marked incidence of meningitis among the casualties of war, suggested that investigations be directed toward the possibility of treatment of these infections. The difficulties in the treatment of these meningitides have been due, aside from the extreme virulence of these pyogenic organisms within the human meninges, to the inability to obtain specific sera and to the technical obstacles to be overcome in reaching the organs involved. So far, therapeutic measures have been applied along two main lines. The first of these is credited to Leonard Hill (5), who wrote (p. 29): "Finally it is suggested that in such a pathological condition as meningitis, irrigation of the meninges might be employed. The operation could be as easily and safely carried out as that of irrigation of the peritoneum." The other therapeutic procedure, advocated by Franca (4), Wolff (12) and others involves freeing the canal of as much pus as possible by lavage, with subsequent injections of bactericidal chemicals into the subarachnoid space. Neither of these methods have as yet been attended by great success but until more specific therapy is available, the indications are that for the present, treatment must depend on the improvement and modification of these general measures.

In the early phases of the work, the method of meningeal irrigation was used for the purpose of determining the toxicity of certain antiseptics within the nervous system. The results ob-

tained by the direct injection into, and by irrigation of, the subarachnoid space by such antiseptic agents, have been made the subject of a separate communication (Wegeforth and Essick (11)). It is purposed here to record experiments dealing with the irrigation of the meninges by simple salt solution and by Ringer's solution, for the sodium salt alone is definitely toxic while the "physiologically balanced" solution is harmless. Furthermore, the results of washing out of the subarachnoid space by a bland solution in the presence of an otherwise fatal infection, are presented in detail.

METHOD

The animals used in all of the experiments were adult cats. These were anesthetized with ether, either by the intratracheal method, or by cone, and the usual precautions were taken to prevent operative infection. In the earlier experiments the irrigation was limited to the spinal canal. For this the first puncture needle was inserted into the subarachnoid space through the occipito-atlantoid ligament (cf. Wegeforth, Ayer and Essick (10)), and the second in the lumbar region, either through the lumbo-sacral ligament or between the fourth and fifth lumbar vertebrae. Between these needles fluid could be passed through the spinal subarachnoid space in either direction, though as a rule the descending route (from cervical to lumbar) was selected. Later, in order to include the cerebral meninges in the irrigation, needles were introduced into the subarachnoid space in the vertex area; from there the flow could be conducted either to a needle inserted through the occipito-atlantoid ligament (resulting in a cerebral irrigation) or to a lumbar needle (washing out the entire subarachnoid space). In order to introduce the vertex needle, an opening was made through the skin, galea and skull by a small perforator, having the diameter of the needle to be subsequently inserted. To prevent injury to the brain, the perforator was provided with a shoulder which permitted it to penetrate only slightly beyond the inner table of the skull. It was found that the needle could best be inserted into the subarachnoid space of the brain if the perforation were made over the posterior parietal region about one-half inch from the mid-line.

The needles used were either 17 or 18 G. and were provided with a snug fitting obturator. To prevent obstruction of the opening of needle by tissue strands, the vertex needle was provided with a fenestration on either side near the tip. When in place the needles did not require any additional support other than given them by the tissues through which they passed. The irrigating apparatus consisted merely of a glass reservoir of adjustable height, and of the necessary tubing. The pressure of the irrigating fluid was maintained between 300 and 400 mm. of water. The temperature of the fluid as it entered the meningeal cavity was found to be of great importance and precautions were taken to maintain it at constant body temperature. This was accomplished by warming the fluid in the reservoir to approximately the desired temperature by means of a carbon electric bulb, and a more accurate adjustment of temperature was made just as the fluid entered the meninges. A glass "T" tube was inserted into the system close to the needle. In the perpendicular arm of this tube a thermometer was secured by a rubber cuff, which also prevented leakage. The horizontal portion of the glass-tube was wrapped with a few coils of nichrome wire, through which an electric current, controlled by a resistance coil, was passed. By means of this device the temperature of the irrigating fluid could be maintained at any desired level. With the exception of the thermometer, the entire apparatus could be sterilized by boiling.

IRRIGATIONS OF THE SUBARACHNOID SPACE IN NORMAL ANIMALS

As soon as the necessary technical skill in the procedure of irrigation of the subarachnoid space was acquired, experiments were devised to demonstrate the extent of the distribution of the irrigating fluid. For this purpose carbon granules (lamp black or india ink) were added to the fluid in the reservoir, the routine irrigation carried out, and the nervous system and meninges examined after fixation. After a spinal irrigation (needle in lumbar and occipito-atlantoid regions) the whole spinal subarachnoid space was found to be filled with the granules, while the basilar cisterns contained a lesser amount, diminishing rapidly in the higher regions. Over the cerebral cortices practically no gran-

ules could be made out. With the needle inserted in the vertex (cerebral irrigation) the distribution of granules on the side of the needle was quite uniform and covered the major portion of the hemisphere, while the contralateral hemisphere was covered over about two-thirds of its extent. The basilar cisterns were everywhere filled with the carbon granules. Similar results were obtained by the addition of Prussian blue granules to the irrigating fluid. With both of these insoluble particles, the distribution post mortem represented only a minimal spread for the irrigating solution, for many of the granules are necessarily mechanically caught in the meshes of the arachnoid. Even with such limitations, a very wide-spread distribution of the irrigating solution throughout the subarachnoid space is indicated.

The same technical procedure of irrigating with suspensions of granules was carried out on cadavers. The distribution of the carbon particles in the fluid throughout the human meninges was very extensive and similar in every way to the spread recorded for animals. These results, both in man and animal, indicate that by irrigation within the subarachnoid space, an extensive portion of the central nervous system is directly reached.

Irrigations with physiologically balanced salt solutions

For a physiologically balanced salt solution, the formula recommended by Locke was first employed. Two objections to the use of this solution were apparent when it was subjected to sterilization in the autoclave, namely, the formation of caramel from the glucose and the precipitation of calcium carbonate. For this reason the sodium bicarbonate and the glucose were omitted from the original formula. The final irrigating solution for these experiments on cats was really a modified Ringer's solution, made up as follows: NaCl, 0.9 per cent; CaCl_2 , 0.024 per cent; KCl, 0.042 per cent. Irrigation with this modification at once demonstrated that the meninges of the cat could be irrigated throughout their entire extent, without causing any noticeable change in the animal, either during or subsequent to, the operation. Of the ten animals so treated, none died. One of the earlier animals,

however, died shortly after having received a cerebral irrigation lasting one hour, during which 221 cc. of the fluid had been run through. Sodium bicarbonate was being used in the irrigating solution at this time and there was a marked precipitate of calcium carbonate present in the solution used; the absence of available calcium may have been the cause of death in this case.

Irrigations of the spinal canal alone were carried out on four cats. These experiments were all very satisfactory and none of the animals showed any evidence of toxicity or effects of trauma. The one disadvantage in these irrigations, however, was that the flow was always slow, and in none of the experiments was a large quantity of fluid obtained at the needle of exit. The largest amount passing through the spinal meninges was 106 cc., this being the result of an irrigation of one and one-half hours. In all of the other experiments the irrigation was from the vertex to the occipito-atlantoid ligament. These were extremely well borne and had an advantage over the spinal irrigation in that a more rapid flow of the fluid was obtained. The usual irrigation was one hour; during this time the amounts of fluid collected varied from a few hundred to over a thousand cc. The following protocol of one of the cerebral irrigations illustrates quite clearly the ease and harmlessness of the procedure:

Cat 77. Male adult

November 16, 1917. 2.45 p.m. Intratracheal ether anesthesia. Vertex puncture followed by occipito-atlantoid puncture—clear cerebro-spinal fluid obtained from both needles. Irrigation apparatus arranged for irrigation with Ringer's solution at 400 mm. water pressure, from vertex to occiput.

3.02 p.m. Both needles dripping freely. Irrigation started.

3.17 p.m. Flow is good and fluid clear. Total 394 cc. run through.

3.32 p.m. Flow is good and fluid clear. Total 592 cc. run through.

3.47 p.m. Flow is good and fluid clear. Total 817 cc. run through.

4.02 p.m. Flow is good and fluid clear. Total 1027 cc. run through.

Irrigation stopped at 4.02 p.m. Animal in fine condition. Recovery from the anesthesia normal.

November 17. Animal is up and around. Drinks milk readily. Apparently perfectly well.

November 18. Cat is in fine shape and can not be distinguished from a normal animal. No signs of weakness.

The animal remained under observation until December 7, during which time it never showed any harmful effects of the irrigation.

These observations indicate that the subarachnoid space of a cat may be safely irrigated with Ringer's solution of proper composition; no ill-effects from the procedure are noted.

Irrigations with normal sodium chloride solutions

In striking contrast to the normal behavior of animals subjected to meningeal irrigations with Ringer's solution were the extreme toxic symptoms manifested by cats in which similar irrigations were carried out with normal sodium chloride solutions. The animals in the former series lay perfectly quiet during the irrigation and after recovery from the anesthesia showed no ill-effects; those receiving sodium chloride alone exhibited evidences of toxicity of the solution during the course of the irrigation. The toxicity manifested itself by twitching of various muscle groups (especially those of the ears) and these initial irritative movements were followed by respiratory difficulties. The toxic effects were often so marked that death ensued before the projected irrigation of one hour's duration could be completed. The animals which survived the experimental procedure died sometimes a few hours later, while others lived for several days. During these periods the clinical course was rather typical and was characterized by frequent convulsions and attacks of acute mania, during which there would be erection of the hair, bushing of the tail, dilatation of the eyes, aimless biting, and an uncovering of the claws. In only three experiments did the animals survive the simple spinal irrigation with sodium chloride solutions without showing such evidence of cortical irritation; four others died from the respiratory failure during or shortly after the completion of the irrigation. Two of the three animals surviving were found dead in the cage on the second and eighth days respectively, following the irrigations. A pneumonia with empyema, sufficient to account for death, was found

at autopsy in both animals. The cat dying on the second day had received an irrigation of thirty minutes with 187 cc. of 1 per cent sodium chloride, but in the other, owing to technical difficulties, the procedure was stopped at the end of fifteen minutes when only 7 cc. of the salt (not enough to cause symptoms) had been run through. The remaining cat in this series, after having its spinal canal washed for over an hour with sodium chloride solution (106 cc. of 0.7 per cent) recovered from the anesthesia but subsequently had attacks of mania, followed by partial paraplegia for several days. It eventually recovered and became normal. The protocol of this animal is given:

Cat 22. Adult female

September 25, 1917. 3.05 p.m. Ether. Lumbar puncture—clear fluid obtained. Occipito-atlantoid puncture—clear fluid also. Irrigation apparatus for salt solution (0.7 per cent NaCl) at 400 mm. water pressure connected with cervical needle.

3.20 p.m. Irrigation from occipital to lumbar needle started.

3.40 p.m. Regular spasmodic twitching movements of hind part of body. 19 cc. fluid collected at lumbar needle. Flow is slow but steady.

4.52 p.m. Cat has had marked respiratory difficulties. Spontaneous respiration has started again after practically stopping. For a few minutes thereafter respiratory irregularities.

5.00 p.m. Irrigation ended. 106.6 cc. obtained from the lumbar needle during experiment.

5.30 p.m. Animal having marked spasms and prolonged convulsions associated with beating movements of hind legs. Is furiously insane and is biting self.

September 26. Cat shows evidence of an acute mania but is sitting up. Drags hind quarters when it walks, but hind legs are not entirely paralyzed.

September 27. Paralysis of hind legs still marked but recovery is taking place.

September 29. Animal is improving quite rapidly but is still very weak in both hind legs.

October 8. Animal in excellent condition; returned to large cage with stock animals although there is some stiffness still present in the hind legs.

January 2, 1918. Animal is normal and active.

The experiments in which irrigations of the cerebral meninges were made with the simple saline were very similar in their results to those just given. In this series, of the nine animals used, six died either during the irrigation or within an hour afterwards; they all showed the same evidences of neuro-muscular irritation and respiratory collapse. The three animals which survived all had attacks of acute mania with convulsions within a few hours following the operation. Complete recovery took place in two of these, but the third remained definitely sick and died at the end of nine days. The amount of fluid obtained at the needle of exit in the immediately fatal cases averaged 320 cc., and the duration of the irrigation was from 15 minutes to one hour. In two of the cases which survived, the amount of fluid obtained was 293 cc. and 400 cc., respectively, and the procedure lasted one hour in each case. The irrigation in the third case was intentionally interrupted at the end of thirty-four minutes when only 112 cc. of fluid had been recovered. At this time the toxic effects of the salt had become manifest, as shown by the twitching of the ears and legs; the irrigation was interrupted to ascertain if maniacal seizures, characteristic for the other animals, could be avoided. The cat, however, showed the same effects as those seen in other experiments, the periods of mania lasting over several days. The protocol of this experiment shows very well the immediate effect of the salt and the gradual return to normal:

Cat 646. Adult female

May 17, 1918. 2.50 p.m. Ether. Vertex puncture—fluid slightly blood-stained. Occipito-atlantoid puncture—fluid clear. Irrigation apparatus arranged at 350 mm. water pressure and reservoir filled with 0.9 per cent NaCl solution.

2.56 p.m. Irrigation begun from the vertex to the occipito-atlantoid needle.

3.25 p.m. Animal showing signs of irritation—twitching of the ears.

3.28 p.m. Twitchings are present in the muscles of the neck and hind legs.

3.30 p.m. Experiment terminated. The flow of fluid has been steady and 112 cc. were collected at the cervical needle. After recovery from the anesthetic animal developed violent convulsions, lasting for several minutes at a time.

May 18. Cat is sitting up but is weak. When disturbed she becomes maniacal, running aimlessly about the cage.

May 19. Seizures of acute mania persist; animal looks wild.

May 20. There is an evident weakness of the hind legs, made apparent by clumsiness in landing when animal is dropped. The maniacal outbursts were not seen after May 21, but the weakness of the hind legs persisted until May 30. After this, animal's condition was normal until the end of the observation on June 14.

The irrigation then, of the cranial or spinal subarachnoid space with simple saline solutions results in frequent death of the animal from respiratory failure during the procedure. While the saline is passing through the meninges frequent neuro-muscular phenomena of irritation are shown. Animals not immediately dying from the irrigation usually exhibit extreme convulsive and maniacal attacks, the irritability enduring at times for several days. Partial paraplegia, weakness, and ataxia are frequent sequelae of the procedure. No essential difference in reaction was noted when various percentages from 0.7 to 1 per cent sodium chloride in distilled water were used.

Irrigations with partially balanced salt solutions

The marked differences in the results obtained after irrigation with Ringer's solution and with normal sodium chloride solutions led to a few observations to determine the effect upon the central nervous system of the removal of the potassium or calcium radical from the Ringer's formula. Accordingly, two series of experiments similar to those just described were conducted; in the first of these the irrigating fluid consisted of 0.9 per cent NaCl and 0.024 per cent CaCl_2 , and in the second 0.9 per cent NaCl and 0.042 per cent KCl.

In the first of these groups in which the potassium salt was omitted, four animals were used. The irrigation in each case extended over a period of one hour and the amounts of fluid,

collected at the needle of exit, were 627, 780, 1275, and 1533 cc. respectively. Aside from a little sluggishness of movement exhibited the following day by two of the cats, none of them showed any noticeable toxic symptoms and so far as could be determined the results of the irrigations were the same as those obtained after the use of Ringer's solution. All of the animals were kept under observation for one to three weeks.

The results obtained when the calcium radical was omitted were quite different. There were five experiments in this series and in three of them, the cats were killed by the effects of the solution before the completion of the hour's irrigation. A fourth animal survived the operation only half an hour. In all of these four observations, the same toxic manifestations, seen in the sodium chloride experiments, were present and death seemed to be the result of cardio-respiratory failure. The remaining animal of the series, after recovering from the anesthetic, suddenly developed a tetany-like shaking of the whole body, changing into epileptiform convulsions with retraction of the head, neck and back. These attacks continued for about ten minutes, after which the cat slowly became quiet. By the following day the animal showed signs of weakness which gradually disappeared, and for the remaining period of observation (twelve days) it was apparently normal.

The findings, detailed in the foregoing paragraphs, coincide closely with the well-known need of organisms for a properly balanced solution of sodium, potassium and calcium. The early observations leading to this conception were presented by Sidney Ringer (8) in a number of papers published in the *Journal of Physiology*. Loeb (7), however, demonstrated the applicability of the idea of a "physiologically balanced" solution to many of the lower forms of animal life. The necessity of such a balance in the salts in solution was shown by Cushing (1) to hold for the nerve-muscle preparation in the perfused frog; here the toxicity of the pure sodium-chloride solutions apparently affected the nerve-muscle junction. The importance of the calcium radical has been emphasized by many writers in relation to many activities in the organism (cf. Howell (6)). Our results indicate that

the omission of the potassium fraction is by no means as important as that of the calcium in irrigations of the subarachnoid space, but further experimentation is required for final substantiation of this conception. It is quite strange that Falkenheim and Naunyn (2), and Hill (5) did not notice the toxicity of the pure sodium chloride solutions upon the central nervous system for the experiments of these investigators had to do with the subarachnoid introduction of fairly large quantities of the simple sodium chloride solution. The toxicity of the normal saline should also have been brought out in those experiments on animals in which anesthesia was produced by the maintenance of an extreme intracranial pressure by the solution. It is felt, however, that the toxicity of pure sodium chloride solutions, on subarachnoid lavage, is amply demonstrated by the experiments recorded.

IRRIGATION OF INFECTED MENINGEAL SPACES

Having demonstrated in the normal cat that the entire cerebral and spinal subarachnoid spaces may be safely irrigated with a properly balanced physiological salt solution, the application of the procedure as a therapeutic measure for meningitis in the cat was investigated. The animals used in these experiments were all given inoculations of bacteria in amounts far above the minimal lethal dose, by either one of the two methods. By the first of these methods the organisms were injected directly into the subarachnoid space through lumbar or occipito-atlantoid puncture-needle. The other method concerned the intravenous injection of the organisms followed by release of cerebro-spinal fluid (cf. Weed, Wegeforth, Ayer, and Felton (9)). In both ways a typical meningitis was caused, leading in the routine experiment to death of the animal within forty-eight hours. The organism used in practically all of these observations was the *B. lactis aerogenes*, a strain which has been found useful in this laboratory for the production of a uniformly fatal meningitis (cf. Felton and Wegeforth (3)). In order to secure absolutely comparable results, at least one, and in many cases two or more cats, were used as controls for the irrigation. The control animals

were given a similar dose of the same culture as those irrigated, were subjected to the same procedures, and were kept subsequently in the same way. In this series of experiments the procedure of washing out the meningeal spaces was usually begun in from two to four hours after inoculation of the animal with a fatal dose of the infecting organism, and the operation was carried out but once in any one animal. The direction and extent of the irrigation varied in the different experiments; in the majority of cases the flow was from a vertex needle through the cerebral subarachnoid space and out through the occipito-atlantoid or lumbar needle. The operation was continued for approximately thirty minutes; in this time from 50 to 500 cc. of fluid were recovered from the draining needles. The irrigation fluid was run in at a temperature slightly above that of the body, under a pressure of from 300 to 500 mm. of water. Cultures of the irrigating fluid as it dropped from one of the lower needles were taken at the beginning and throughout the course of the irrigation. In the typical cases, cultures taken at the beginning of the experiment were positive for the infecting organism; a second culture of the fluid, taken in ten minutes might be either positive or negative, while all subsequent cultures would give no growth. In other cases, all of the cultures would be positive throughout the time of irrigation. In addition to these cultures, specimens of fluid were taken at the same periods for chemical analysis. It was found that during the routine irrigation, the sodium chloride and globulin content as well as the gold reaction in the samples, were unaffected so that the fluid coming through the subarachnoid space was apparently unaltered in its course. Table 1 well illustrates these statements.

Experiments of the type included in table 1 have indicated the possible therapeutic value of a single irrigation with Ringer's solution. The single washing-out of the subarachnoid space has in many cases prolonged the life of the animal, as judged by comparison with the period of survival in the control. In the larger number of series, the irrigation has benefitted the experimental animal, especially in the instances in which the initial positive cultures became negative as in table 1. It is felt that this change

from the positive to a negative culture indicates that a larger number of organisms have been washed out, but it is not believed that all of the infecting bacteria are removed. The procedure probably reduces the organisms available for infection of the meninges and allows the animal's tissues and fluids opportunity for defensive reaction.

In two cases the prolongation of life was so great that an apparent cure of an otherwise fatal experimental meningitis seemed to have been effected by means of a single irrigation with Ringer's solution. The first of these cats was given an intravenous injection of 0.25 cc. of a broth culture of *B. lactis aerogenes* with

TABLE 1
Cat 667; adult male; May 23, 1918

TIME	AMOUNT FLUID	CULTURE LUMBAR	CULTURE OCCIPITO- ATLAN- TOID	GLOBU- LIN PER LITER	CHLO- RINE PER CUBIC CENTIME- TER	REMARKS
<i>p.m.</i>	<i>cc.</i>			<i>grams</i>	<i>grams</i>	
2.21	2	Pos.	Pos.			Initial inoculation with <i>B. lactis aerogenes</i> two hours previously. Irrigation with Ringer's solution from vertex to occipito-atlantoid and lumbar needles. Temperature of fluid constant at 102°F. Pressure 410 to 320 mm. H ₂ O. Irrigation begun at 2:21 p.m. Ended 2.50 p.m.
2.27	142			None	0.0060	
2.36	267	Neg.	Neg.	None	0.0060	
2.45	453			None	0.0060	
2.49	558	Neg.	Neg.	None	0.00587	
2.50	638			None	0.00595	

release of cerebro-spinal fluid in two minutes. Irrigation with Ringer's solution was begun four hours afterwards; about 200 cc. was washed through from vertex to occiput. The two initial cultures of this irrigating fluid were positive and the rest were negative. The animal recovered and remained normal for thirty-six days. Death occurred from another cause; at autopsy normal meninges and central nervous system were found. The control animal, subjected to similar inoculation and release of cerebro-spinal fluid died in 27 hours. At autopsy this control cat showed an extensive leptomeningitis.

The protocols of this series are as follows:

Cat 492. Adult male

April 11, 1918. 12.05 p.m. Ether. Intravenous injection of 0.25 cc. twenty-four hour broth culture *B. lactis aerogenes*. Two minutes later release of 1.5 cc. cerebro-spinal fluid by puncture of the occipito-atlantoid ligament.

4.14 p.m. Irrigation of the meninges for half hour with 202 cc. Ringer's solution. Irrigation from vertex to occiput only. The first two cultures were positive for *B. lactis aerogenes*, whereas the last two were negative.

8.30 p.m. Animal in good shape, sitting up drinking water.

April 12. Cat in very good condition. Only abnormality is a slight weakness in hind legs.

April 20. Animal's condition has remained excellent. Spinal fluid today shows no increase in cells or proteid.

May 17. Animal has been normal and active until two days ago, when it was suddenly taken sick and died.

Autopsy. Both gross and microscopic shows no lesion of the meninges or evidences of inflammation within the central nervous system.

Cat 490. Adult female

April 11, 1918. 11.40 a.m. Ether. Intravenous injection of 0.25 cc. same broth culture *B. lactis aerogenes*. Two minutes later release of 1.5 cc. cerebro-spinal fluid by puncture of the occipito-atlantoid ligament.

8.30 p.m. Cat is very sick. Crouches in cage with head down. Retraction of head causes no rigidity.

April 12. 9.30 a.m. Cat is very sick; lies in cage with head drooping; can hardly be roused. Retraction of head causes marked extensor rigidity. Gross clonus of head.

2.30 p.m. Animal moribund, no anesthetic required for puncture of occipito-atlantoid ligament. Smear of fluid shows numerous pus cells, with bacilli both free and phagocyted. Cat died a few moments after fluid was obtained.

Autopsy. Acute generalized leptomeningitis.

Two other animals given each 0.25 cc. of the same culture intravenously on April 11 and subjected to lumbar puncture two minutes later were both dead the next day with meningitis.

The second case of apparent clinical cure died finally after fourteen days with meningitis which was diagnosed only by pathological examination. In this animal the infection was caused by an intravenous injection, followed by release of the cerebro-spinal fluid in three hours. A single irrigation was done routinely and 172 cc. was run through. The control in this series died in 64 hours. The protocol of this experiment with its control are given:

Cat 658. Adult female

May 22, 1918. 11.10 a.m. Without ether. Intravenous injection 0.25 cc. twenty-four hour broth culture *B. lactis aerogenes*.

2.20 p.m. Ether. Release of cerebrospinal fluid (1.5 cc.) by puncture of the occipito-atlantoid ligament. Immediately thereafter the meninges were irrigated for twenty-five minutes with 172 cc. Ringer solution.

May 23. Cat appears to be comfortable, a little weak but not groggy. Head held up in fine shape. Retraction of head causes no trouble.

June 4. The animal has remained in excellent general condition. A little weakness in hind legs, shown only when cat is dropped. Eats normally.

June 5. Animal found dead in cage.

Autopsy. Mild inflammation of the meninges.

Cat 659. Adult male

May 22, 1918. 11.15 a.m. Without ether. Intravenous injection 0.25 cc. same broth culture *B. lactis aerogenes*.

3.50 p.m. Ether. Release of cerebrospinal fluid (1.5 cc.) by puncture of the occipito-atlantoid ligament.

May 23. Cat is very sick and weak, lies on side. Unable to stand up. Retraction of head causes no discomfort. Wobbly and groggy.

May 24. Cat is down on side, cannot stand. Moribund by afternoon.

May 25. Cat found dead in cage.

Autopsy. Acute exudative leptomeningitis of cord and brain.

Of probably greater significance than the occasional cure is the prolongation of life, effected in many of the experimental series by a single washing of the meninges with this bland solution. Thus, in fifteen series of cats, there has occurred a lengthening of the period of survival after a single irrigation of about 100 per cent. In one of these series the control died in twenty-four hours, while the irrigated animal lived fifty-four hours. In another the controls died in twenty-four and forty hours, respectively; the irrigated animals died in forty and fifty-one hours, that living only 40 hours was technically a poor irrigation. Similarly, after the subarachnoid injection of 1 cc. 1-1,000,000 broth culture of *B. lactis aerogenes*, the control was found dead on the third morning while the irrigated animal did not die until the fifth morning. With still another group of animals, the control died in five hours and the two irrigated cats lived until the next morning. The following protocols well illustrate this class of experiments:

Cat 594. Adult female

May 13, 1918. 11.09 a.m. Ether. Intravenous injection 1 cc. (1-100) twenty-four hour broth culture of *B. lactis aerogenes*. Two minutes later, occipito-atlantoid puncture with release of 1 cc. clear cerebro-spinal fluid.

2.45 p.m. Meninges irrigated from vertex to occipito-atlantoid and lumbar needles with 519 cc. of Ringer's solution. Cultures of the fluid taken at beginning of procedure were positive, but those taken later during irrigation were negative. Animal left table in good condition.

5.00 p.m. Animal is quite excitable but in good shape.

May 14. 9 a.m. Normal and active.

5.00 p.m. Animal has commenced to show signs of meningeal irritation; is having convulsions with legs stiffly extended and back flexed.

May 15. 9.00 a.m. Dead in cage.

Autopsy. Acute diffuse meningitis.

Cat 592. Adult male

May 13, 1918. 11.00 a.m. Ether. Intravenous injection 1 cc. (1-100) same broth culture of *B. lactis aerogenes*. Two minutes later occipito-atlantoid puncture with release 1.5 cc. clear cerebro-spinal fluid.

5.00 p.m. Active and apparently normal.

May 14. 9.00 a.m. Down on side having convulsions. Retraction of head causes convulsions.

2.30 p.m. Dead in cage.

Autopsy. Acute generalized meningitis.

The prolongation of life as recorded in the foregoing protocols is really not extreme enough to warrant extensive conclusions. Individual variations might well account for a disparity in the period of survival after such inoculations, and a realization of this is necessary for the proper interpretation of the findings. The period of prolongation of life in animals with an otherwise fatal meningitis has, in fifteen different series, been lengthened approximately 100 per cent by a single irrigation with a bland solution. Summary of these cases indicates that the procedure is not without promise in infections for which no specific treatment is available.

In still another series of experiments the control and irrigated animal showed the same clinical reactions, both dying within a short time of one another. It can be said for this series that the

irrigation did no harm. It was not hoped the use of such simple solutions would effect complete cures, but the results demonstrate that the single procedure is of some service in removing at least a portion of the bacteria. The washing-away of some of the toxic products of bacterial growth seemed also of service, for the irrigated animals were usually more nearly normal for the next twenty-four hours than the controls.

The technical difficulties encountered in this series of experiments was great and a high mortality was obtained during the preparation for, or progress of, the irrigation. The initial mortality was due to the extreme toxicity of the infected animals, many of which were in a state of collapse when the operation was begun. Frequently administration of the anesthesia alone was sufficient to cause death. On the other hand, animals surviving the irrigation were in a better shape at the end of twenty-four hours than the control animals which had not been subjected to irrigation, so that the hastening of death by the technical procedure was more than balanced by the prolongation of life in the remaining cases.

SUMMARY

1. Irrigations of the spinal and cerebral subarachnoid spaces are well tolerated by cats if the irrigating fluid is composed of sodium chloride, potassium chloride, and calcium chloride in proper proportions (modified Ringer's solution). If, however, the irrigation be done with isotonic solutions of sodium chloride alone, various toxic effects become apparent. Many of these animals die during or immediately after the irrigation; if this immediate toxicity is survived, convulsive seizures and acute mania are almost invariable. Recovery from such attacks is frequent.

2. Single irrigation with modified Ringer's solution of infected meningeal spaces has prolonged the life of the animals, as compared with controls. The period of survival in many cases has been doubled as a result of this washing-out of the infected meninges. Multiple irrigations have not been attempted.

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THE EFFECT OF SUBARACHNOID INJECTIONS OF ANTISEPTICS UPON THE CENTRAL NERVOUS SYSTEM

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Subarachnoid injections of antiseptic agents have been advocated by Franca (7) and Wolff (20) as a means of combating infection of the subarachnoid space. The use of such chemicals was advised not only in the meningococcic cases where specific therapy is of utmost service but in the other types of infection where serum treatment has not yet been developed. It is the object of this communication to give the results of experimental work dealing with the subarachnoid injection of antiseptics. Time has not permitted the investigation of as large a series of these agents as would be desired, nor have sufficient experiments been completed with certain of the antiseptics. A method of irrigation, similar in part to that first devised by Leonard Hill (12) has been employed to test the initial toxicity of certain of the solutions; this method promises much in the pharmacological investigation of the subarachnoid space. Some phases of this work have been so significant in result as to justify certain generalizations regarding the subarachnoid injection of antiseptics. For it is apparent that chemical bodies, applied directly to the central nervous system, exhibit a much greater initial toxicity than when applied elsewhere in the body; microscopical examination of the central nervous system reveals in these cases anatomical changes which persist after the toxicity of the antiseptic has subsided.

CLINICAL REACTIONS OF ANIMALS TO SUBARACHNOID INJECTIONS
OF CHEMICALS

Chloramine. The interest recently devoted to the treatment of local infections with solutions liberating chlorine, and the use of these solutions in wounds of the central nervous system, naturally directed investigation to the possible employment of these chlorine derivations in the meninges. Accordingly, the toxicity of such solutions for the central nervous system was tested by direct injections of varying dilutions and amounts into the subarachnoid space. It was found that 5 cc. of a 0.125 per cent solution of chloramine, (p. toluene-sodium-sulphochloramide) when injected into the cisterna cerebello-medullaris of adult cats, did not kill the animal until after three days. The injection of 1 cc. of a 1 per cent solution into the lumbar region caused a violent clinical reaction; 2 cc. of dilution of 1-1000 by the same route, however, did not apparently affect the animals. This led to the trial of the chemical as a therapeutic agent for irrigating the meninges. Accordingly, irrigations were carried out from the occipital to the lumbar regions by the method used in this laboratory (Weed and Wegeforth (18)). In these experiments chloramine was added to the Ringer's solution in varying concentrations. In the first of these observations, the washing-out of the space was done as usual with the Ringer solution, but at varying intervals during the irrigation, a small amount of chloramine was shunted into the irrigating system. At a definite time after the introduction of this antiseptic to the irrigating fluid, simple Ringer's solution was substituted. In later experiments, continuous irrigations were made with solutions containing definite percentages of chloramine without alternating with Ringer's solution. This experimental procedure was uniformly fatal until a dilution of $\frac{1}{512}$ per cent chloramine was reached, the animals dying either immediately or within the course of twenty-four to forty-eight hours. After such irrigations with chloramine all showed symptoms of a profound toxemia, associated with frequent convulsions, maniacal seizures and subsequent paralysis. One of four animals, subjected to irrigations of the

cerebral meninges from vertex to the occiput with Ringer's solution containing $\frac{1}{512}$ per cent chloramine, recovered, while the others died during the procedure. The irrigation in the cat surviving lasted one hour, during which time 505 cc. of the solution were run through. Later, two irrigations with higher dilutions ($\frac{1}{1024}$ per cent) of the chloramine in Ringer's solution were done; the cats lived eight and ten days, respectively.

Although the early experiments with fairly high dilutions killed the animals, the results were not unexpected and the fact that a point in dilution could eventually be reached at which the bactericidal qualities of the solution were preserved and the toxicity for the animals lost, indicated a possible therapeutic value. However, an investigation of the pathology of the central nervous system of the animals, which were apparently unaffected clinically by the drug, made it imperative to study the problem from a different point of view—namely, the ultimate pathological changes. Accordingly, subarachnoid injections of small amounts of chloramine in Ringer's solution were made and the cerebro-spinal fluid examined each day thereafter. Within twenty-four hours after such injection, the number of white blood cells and the globulin content of the fluid showed enormous increases. From this maximum at twenty-four hours a gradual decrease in cell-count and globulin occurred, reaching a normal about the fifth or sixth day. But meanwhile, with the abnormality of cerebro-spinal fluid decreasing, the condition of the animal did not improve but became progressively worse.

The protocol of a typical experiment of this kind with a subarachnoid injection of a 1 per cent solution of chloramine by lumbar needle, showed the return to normal of the cerebro-spinal fluid as judged by cell-count and globulin-content, but clinically the animal did not improve.

Cat 1445. Large male.

November 27, 1918. 2.20 p.m. Ether. Lumbar puncture, 6 drops of clear fluid obtained, subarachnoid injection of 1 cc., 1 per cent chloramine solution. Immediately thereafter, animal developed acute mania. Prolonged for several minutes.

November 28. Almost total paralysis of left hind-leg, weakness in front legs. By occipito-atlantoid puncture turbid cerebro-spinal fluid, containing 7 grams of globulin per liter and 4480 white cells.

November 29. Almost total paraplegia. Cerebro-spinal fluid slightly turbid, containing 10 grams of globulin and 980 white cells.

November 30. Same condition of partial paraplegia. Cerebro-spinal fluid almost clear, containing 9 grams of globulin and 610 white cells.

December 2. No change in condition of hind-legs. Cerebro-spinal fluid clear, containing 0.5 gram of globulin and 20 white cells.

Animal remained in condition of practically total paraplegia with gradual decline (retention of urine) until December 11, when it was found dead in cage.

The foregoing experiments dealing with the subarachnoid injection of chloramine solution even in great dilutions, demonstrate a rather high toxicity of the antiseptic within the central nervous system; with sublethal doses, a violent reaction in the cerebro-spinal fluid occurs, manifested by increase in globulin-content and cell-count. The subsidence of the abnormalities in the fluid, have no relation to the clinical course; animals die after several days at a time when analysis of the spinal fluid would indicate that the effect of the drug on the central nervous system had subsided.

Flavine. The employment of acriflavine by urologists (Davis and Harrell (4)) in the treatment of acute urethritis suggested investigations of its toxicity within the nervous system. The drug is a member of the acridine series of compounds, many of which have marked antiseptic properties. One of the first experiments carried out with this chemical consisted in injecting 1.5 cc. of a 1-1000 dilution into the cisterna magna of a cat. Several days later an attempt to obtain fluid from the same animal by puncture through the occipito-atlantoid ligament was unsuccessful, although the needle seemed certainly to be properly inserted. The animal had remained apparently normal since the time of the injection, but it was nevertheless felt that the failure to obtain fluid was the result of some inflammatory change in the meninges, rather than faultiness of technique in perform-

ing the puncture. Accordingly a series of experiments to determine the extent of this reaction within the meninges was instituted. The injections were all made in the lumbar region in dilutions varying between 1-1000 and 1-5000. The effect of these was in many respects similar to that caused by the chloramine, although the initial clinical upset was not so violent nor were the cellular reactions of the spinal fluid as marked. In only one of the cases was the immediate toxicity sufficient to cause death within twenty-four hours. The others living many days, exhibited unmistakable signs of local damage to the central nervous system, such as partial or complete paralysis with occasional loss of sensation in the hind-legs and retention of the urine.

The spinal fluid in such animals, examined daily, showed an initial purulent reaction which gradually became less intense and in a few days disappeared. The globulin-content rose rapidly in the first twenty-four hours but did not drop to normal as soon as did the cell-count. The cerebro-spinal fluid was obtained by puncture of the occipito-atlantoid ligament. The following protocol of a cat receiving 2 cc. of acriflavine (1-5000) into the lumbar meninges, illustrates very well the downward clinical course ending with death on the sixth day, whereas the cell-count of the cerebro-spinal fluid returned to practically normal on the second day.

Cat 1459. Large male

November 29, 1918. 4.30 p.m. Ether. Lumbar puncture, 1 cc. clear fluid obtained. Subarachnoid injection 2 cc. of 1-5000 acriflavine. Immediately after injection the animal breathed heavily and groaned somewhat; later became quiet and lay on side.

November 30. Cat in good general condition. Somewhat weak and ataxic in hind-legs. By occipito-atlantoid puncture 2 cc. turbid fluid, containing 1 gram globulin per liter, and 2800 white cells per cubic millimeter.

December 2. Animal in fair shape. Still ataxic. Cerebro-spinal fluid clear, contains 1 gram globulin per liter and 20 white cells.

December 4. The general condition has remained good but both hind-legs have become very spastic.

The spasticity of the hind-legs changed into an almost complete paraplegia and the general condition became progressively worse until December 6, when the animal was sacrificed.

The experiments with acriflavine, like those made with chloramine, indicates clearly that the study of the cerebro-spinal fluid affords but a poor index of the nature and extent of the changes in the central nervous system, following subarachnoid injections of these chemicals. Only initial acute reaction in the meninges caused by such irritants can be followed, by the changes in the spinal fluid.

Potassium permanganate. Experiments with potassium permanganate demonstrated that a lumbar injection of 5 cc. of a 1-1000 dilution killed almost instantly. An injection of 2 cc. of the same dilution on the other hand was tolerated very well, and aside from a slight weakness in the hind-legs the animal showed clinically little reaction.

Potassium biniodide. Only two experiments were made with this drug. In the first a 2 cc. dose of 1.0 per cent solution by lumbar injection caused immediate death, but the same dose of a 0.5 per cent produced a condition very similar to that noted in the experiments given above and the animal lived two days. During the injection of the drug in this latter case, there was progressive beating of both hind-legs. This was followed by practically a total paraplegia, the animal becoming almost helpless.

Benzoyl alcohol (2 cc. of 3 per cent solution) and *mercuric iodide*. (3 cc. 0.1 per cent solution) were both well tolerated by the animals, which clinically showed no effects of the lumbar injections. *Carbolic acid* (3 cc. of 1 per cent solution) produced a severe initial reaction and left the animal with definitely weak hind-legs and was, in that respect, similar in its action to chloramine and acriflavine. *Silver nitrate* (3 cc. of 1 per cent) killed the animal within twenty-four hours after the subarachnoid injection. The dilutions used in the above experiments were based on rough determination of the bactericidal powers against *B. lactis aerogenes* carried out in vitro.

Lysol. Lumbar subarachnoid injections of 1 per cent solutions of lysol in Ringer's solution were well tolerated by the cat, in amounts not exceeding 2 cc. No clinical manifestations were recorded in animals receiving this dose; they remained normal and active. When, however, such 1 per cent solutions of lysol were irrigated from the occipital to the lumbar region, death ensued after 4 to 5 cc. had been run through. Direct subarachnoid injection into the cisterna magna through the occipito-atlantoid ligament of 2 cc. of the 1 per cent lysol solution resulted in immediate death of the animal from respiratory and cardiac failure. The striking characteristic of these experiments was the fact that total recovery took place, provided that the cat did not die from the immediate toxicity of the drug.

PATHOLOGICAL CHANGES IN THE CENTRAL NERVOUS SYSTEM

The pathological changes in the central nervous system and its meninges as a result of all the chemicals injected into the subarachnoid space, were very similar, differing mainly in degree. When dilute solutions were used the result was confined entirely to the meninges. The structures next affected were the marginal fibres of the spinal cord. When stronger dilutions were used, a large part of the white matter was destroyed without altering the gray matter. After using potassium biniodide, the entire substance of the cord was involved in a destructive myelitis. The changes of the spinal cord, as seen in gross, were confined largely to the site of injection; when injected into the lumbar region, marked pathological changes extended into the thoracic cord and gradually faded as the upper cervical region was approached. The markings, as seen through the dura, were clouded; the membranes looked opaque. Usually the spinal cord was swollen, more markedly in the lumbar region. The brain was entirely unaffected after the lumbar injection.

The fusion of the membranes of the spinal cord was the most constant pathological finding, microscopically. Normally, the nervous tissue shrinks very considerably during embedding, while the membranes contract very little. This results in an appearance similar to figure 1. Here, a large subdural space has

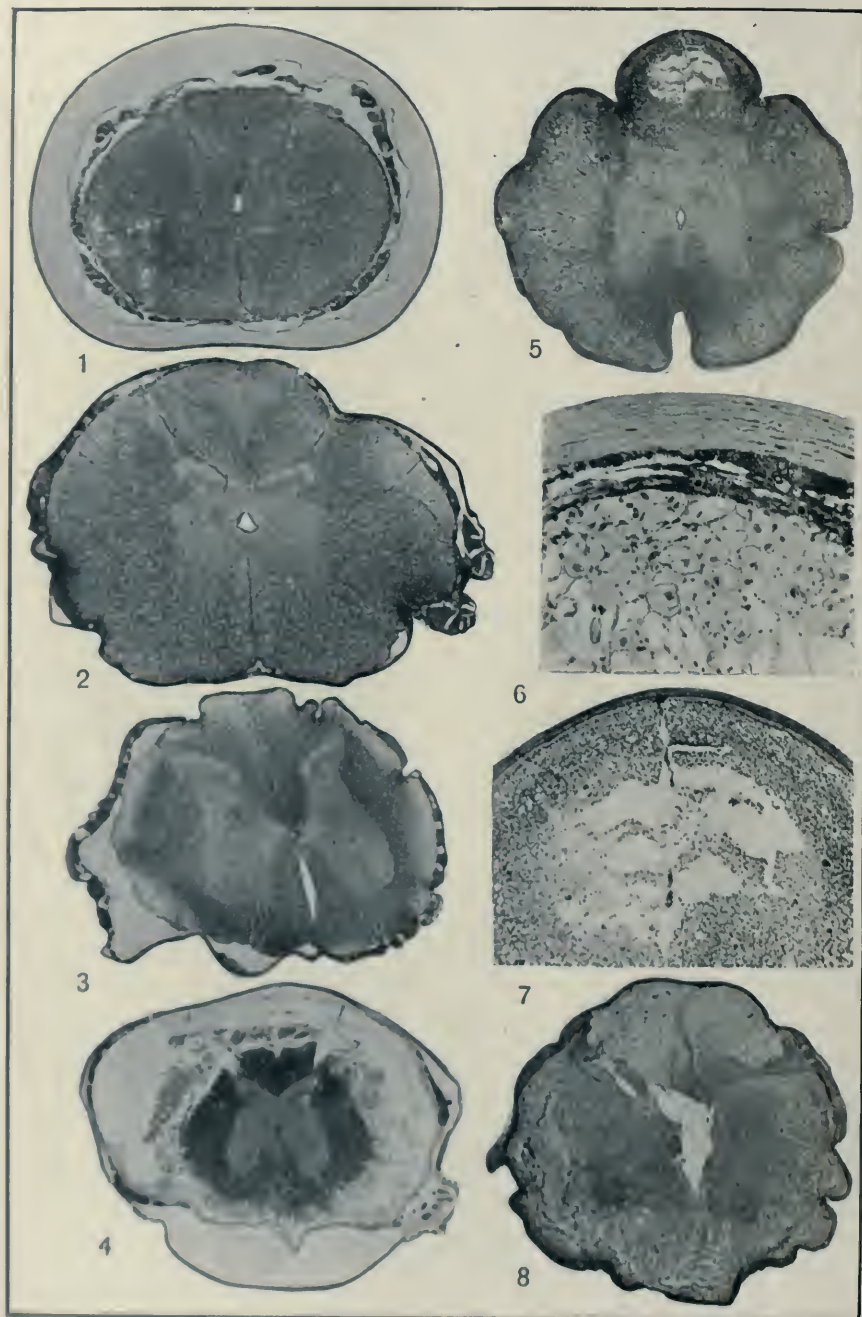


FIG. 1. Photomicrograph. Section showing the normal relations of the spinal cord to the meninges after embedding. In life, the arachnoid membrane is approximated to the dura and the subdural space is relatively not as large as shown in this figure. The shrinkage of the nervous tissue, normally, produces the relations shown in this illustration. Hae-Eosin. $\times 10$.

FIG. 2. Photomicrograph. Section through fourth thoracic segment of a cat, which received, ten days before death, a lumbar subarachnoid injection of 2 cc. of 1-1,000 solution of chloramine. The dura and leptomeninges are tightly adherent to one another. The substance of the cord is entirely unaffected. The wrinkling of the outline has been occasioned by the disproportionate shrinkage of the meninges and the nervous tissue. Hae-Eosin. $\times 11$.

FIG. 3. Photomicrograph. Section through fourth lumbar segment of a cat, which had received, ten days before death, a lumbar subarachnoid injection of 1 cc. of 1-2500 acriflavine. In addition to the fusion of the meninges, the most superficial zones of the nervous tissue have been injured by the chemical, as shown by their lighter staining qualities. A higher magnification of this change is shown in figure 6. During the embedding of the specimen, the matted meninges have been torn away from the spinal cord. Hae-Eosin. $\times 10$.

FIG. 4. Photomicrograph. Section through fourth thoracic segment of a cat, which had received four days before death, a lumbar subarachnoid injection of 1.5 cc. of 1-1000 acriflavine. The central portion of the spinal cord is still apparently unaffected. The outer zones have become so necrotic as to form no firm anchorage for the fused meninges to the cord. Consequently, the dura has assumed an outline more nearly like the normal shown in figure 1. Hae-Eosin. $\times 8$.

FIG. 5. Photomicrograph. Section through twelfth thoracic segment of a cat receiving, two days before death, 2 cc. of a 0.5 per cent solution of potassium biniodide. The close adherence of the meninges to the spinal cord and the corrugations produced by the disproportionate shrinkage of the structures is very well illustrated. The effect of the chemical has almost reached the central gray matter, which shows, microscopically, early signs of degeneration. The cavity in the dorsal region is shown under higher magnification in figure 7. Hae-Eosin. $\times 8$.

FIG. 6. Photomicrograph. Enlargement of the dorsal edge of section shown in figure 3. What remains of the subarachnoid space is here shown, injected with india ink. The lower part of the illustration shows the invasion of the fibre tracts by numerous large macrophages. Hae-Eosin. $\times 190$.

FIG. 7. Photomicrograph. Enlargement of the dorsal area of section shown in figure 5. The accumulation of fluid within the fibre tracts communicates with the perivascular space of one of the penetrating vessels. Hae-Eosin. $\times 40$.

FIG. 8. Photomicrograph. Section through fourth lumbar segment of a cat which had received, the day before death, a lumbar subarachnoid injection of 2 cc. of a 0.5 per cent solution of potassium biniodide. A complete transverse myelitis has resulted, accompanied by a sero-fibrinous exudate throughout the entire substance of the spinal cord. Hae-Eosin. $\times 11$.

been artificially produced by the unequal shrinking of the spinal cord and leptomeninges. Similarly, the arachnoid membrane is farther away from the pia mater than normal because the latter closely followed the nervous tissue in its contraction. Such an appearance does not represent the living relations in any sense, but gives us the technical changes brought about in the normal central nervous system during fixation and embedding.

The first change occasioned by the injection of antiseptics into the subarachnoid space was the elaboration of a coagulable fluid which would clot in a few minutes after withdrawal from the subarachnoid space. A sero-fibrinous exudate subsequently formed in the areas reached by the chemical and caused the dura and arachnoid to be firmly bound together. It was possible to separate them, however, by careful dissection. This adherence was demonstrable microscopically by an attachment of the arachnoid membrane to the inner surface of the dura. The subarachnoid space was matted together so that in section its meshes seemed to be entirely obliterated and a circulation of cerebro-spinal fluid looked impossible (fig. 2).

With the increasing concentration of the antiseptics, not only the membranes became glued together, but the outer laminae of white fibres were affected (fig. 3). Such involvement was first noted as a swelling of the individual elements together with a destruction of the myelin (sometimes accompanied by dilatations of the perivascular spaces with evident distention of the tissue with fluid accumulations (figs. 5 and 7). The distention of the perivascular space was probably the result of a complete block of the intramedullary drainage of cerebro-spinal fluid, but the phenomenon was noted clearly in only one specimen. Large mononuclear phagocytes in great numbers invaded the meninges and those areas of the spinal cord which had been destroyed by the corrosive action of the antiseptic (fig. 6).

The degeneration of the outer zones of the cord is well illustrated in figure 4. Here the contraction of the uninjured structures resulted in the tearing apart of the degenerated portions of the nervous tissue. Even with the degree of destruction shown in this figure, the central gray matter has a remarkably well-

preserved appearance, which might account for the fact that the paraplegias observed clinically were never total and only developed gradually. Injection of potassium biniodide, caused the greatest amount of injury to the central nervous system, for not only were the meninges and the white matter involved, but a total destruction of the central gray of the spinal cord existed in the lumbar and thoracic regions as well (fig. 8). In this specimen portions of the spinal cord at a distance from the point of injection, showed progressively weaker effects, so that in the cervical region, the alterations were similar to those illustrated in figure 3.

The effects of chloramine, flavine, and potassium biniodide give similar pathological changes in the central nervous system when introduced into the subarachnoid space; the differences are of degree only, and dependent upon the concentrations. The initial effect was to produce a thickening of the pia-arachnoidea, and adhesion of the membranes one to another by a sero-fibrinous exudate. This caused an interference with, but usually not a complete block of, the normal pathways of the cerebro-spinal fluid. In greater concentrations the substance of the spinal-cord was attacked locally, the amount of damage being limited to the part directly reached by the chemical. For ten days after the injury, the nerve cells in the central gray matter did not show marked degenerative changes, in spite of the apparent total destruction of the peripheral zones of the spinal cord. In sufficient strengths, a complete transverse myelitis might be brought about. During the first three days of the experiment, there was a marked leucocytic exudate into the subarachnoid space; after this time the number of polymorphonuclear and small mononuclear leucocytes was very small. The proliferation of the arachnoidal tissue furnished a large number of phagocytic cells which infiltrated the meninges and destroyed nervous tissue.

Camus and Roussy (3) studied the pathological effects produced on dogs after the subarachnoid injection of a mixture of fatty acid, nucleinate of soda and talc. The experiments of these investigators, though not intended to have a therapeutic bearing, nevertheless yielded pathological results that were quite

similar in many respects to those found by us after the use of antiseptics. Following such injections, they encountered great difficulty in obtaining cerebro-spinal fluid after forty-eight hours. At autopsy the meninges were demonstrated to be in close adhesion to each other with entire obliteration of the subdural and subarachnoid spaces. In the substance of the cord, moreover, there occurred numerous cavities which were interpreted as an experimental syringo-myelia.

INFLUENCE OF ANTISEPTICS ON MENINGEAL INFECTIONS

Clinically, antiseptics have been used for combatting infections in the spinal meninges for some time. Léri (13) in 1902 injected a few cubic centimeters of 3 per cent boric acid into the spinal canal of one of his patients, and the same year Seager (17) reports the use of injections of 1 per cent lysol in a series of cases of meningitis, treated on the service of Franca during the epidemic at Lisbon the year before. Manges in this country (14) reported success in the treatment of several meningeal infections by the injection of lysol and similarly Glatard (9) recommended its use. Hajeck (10), in some very badly infected cases, resorted to the use of lysol, collargol, and corrosive sublimate, but all of the patients treated with these chemicals died. Collargol has found rather extensive use in the hands of Menetrier and Mallet (15), but Geronne (8), after using this drug twice, found it to cause his patients so much pain that he abandoned it. Electrargol was tried by Aubertin and Chabanier (1) but Boinet (2) using this salt in the treatment of pneumococcus and diplococcus flavus meningitides had 100 per cent mortality. Wolff (20) accepting the teachings of Westenhoeffer concerning the purely local nature of the meningococcus infections of the meninges and seeing in it a similarity to the gonococcal infections of the urethra, recommended intrathecal injections of protargol. Rolleston (16) after having discouraging results with the serum treatment of the epidemic form of meningitis, used soanim for intraspinal injections and finally Halahan (11) resorted to a retrograde irrigation of the meninges with 0.5 per cent carbolic acid.

The therapeutic use of chemicals has been limited in this laboratory almost entirely to lysol, although a few isolated injections were made with one or two other antiseptics during the presence of a meningitis. It was found after a few trials that 2 cc. of a 1 per cent solution of lysol given by lumbar injection into the subarachnoid space of cats was tolerated very well, at least as far as subsequent clinical manifestations could be used as an index. Assuming therefore that doses of this drug in these dilutions would not in themselves cause death and leaving out of consideration for the time being the pathological changes it might cause in the meninges, animals inoculated with lethal doses of *B. lactis aerogenes* were treated under varying conditions in accordance with the method of Franca. This organism was chosen because it was found that injections of the meninges of laboratory mammals caused by it were uniformly fatal (Felton and Wegeforth (5)). Four animals were given direct inoculations of the organism into the subarachnoid space, followed immediately by an injection of 2 cc. of 1 per cent aqueous lysol solution. In two cases (cat 793 and cat 794) the dose of organisms was 0.25 cc. of a dilution of 1-25,000 of a twenty-four hour broth culture, and in the other two (cat 780 and cat 732) a dilution of 1-50,000 was used with the same dosage. All of the animals lived throughout the following day but were found dead on the second morning. Control animals receiving similar quantities of the organism lived as long as those which were given lysol at the time of inoculation, so that there was no evidence that the drug altered the course of the disease.

Four other experiments, in which the subarachnoid inoculation was not followed until several hours later by the injection of lysol, were then carried out. In one of these (cat 805) the dose was 0.25 cc. of a dilution of 1-25,000 of a twenty-four hour broth culture, and three hours later 2 cc. of a 1 per cent aqueous solution of lysol was given intrathecally by the lumbar route. The animal was dead by the following morning. Likewise, a cat (749) receiving the same dose of a 1-50,000 dilution, followed in four hours by an injection of 1cc. of a 1 per cent aqueous solution of lysol was found dead by the following morning. The

control animal (cat 750) died, however, on the second morning. In another series, two cats (760 and 778) receiving 0.25 cc. of a broth culture in a 1-50,000 dilution followed in two and four and one-half hours respectively by an injection of 2 cc. of 1 per cent aqueous lysol solution did not die until the third morning. The control (cat 779) receiving only the organisms lived until the fourth morning. These results showed that the injection of lysol does not prolong the life of a cat given a lethal subarachnoid inoculation. A single experiment (cat 747) was made in which an injection of organisms was given indirectly from the blood stream prior to the subarachnoid injection of lysol. In this animal an intravenous injection of 0.25 cc. of a twenty-four hour broth culture of *B. latis aerogenes* was given, followed in two minutes by the release of the cerebro-spinal fluid through puncture of the occipito-atlantoid ligament (cf. Weed, Wegforth, Ayer and Felton (19)). Four hours later 2 cc. of 1 per cent aqueous lysol solution was given intrathecally by lumbar injection. The animal was found dead in the cage the following morning, autopsy showing a purulent meningitis, in the exudate of which the organisms were found.

In two experiments, after direct subarachnoid inoculations with *B. latis aerogenes*, the meninges were irrigated with Ringer's solution; this procedure was followed immediately by a lumbar subarachnoid injection of lysol. One of these animals (cat 803) died a few minutes after the operation was completed. The other experiment (cat 752), was carried out as follows: In the morning at 11.12 the cat received a lumbar subarachnoid injection of 0.25 cc. of a twenty-four hour broth culture diluted 1-50,000. Four hours later the meninges were irrigated for forty-five minutes from the vertex to the occipital and lumbar regions with 50 cc. of Ringer's solution. At the end of the irrigation 1 cc. of 1 per cent lysol was given through the lumbar needle. Four cultures taken at intervals throughout the irrigation were all positive for *B. latis aerogenes*. The animal left the table in good condition and the following day appeared to be normal. On the second day, however, meningitis developed and the cat was found dead on the fifth morning. The control animal (cat

750) for the experiment received on the same day a subarachnoid injection of the same dose of organisms but received no therapy subsequently. The day following the inoculation the cat had convulsions and on the second morning it was found dead in the cage. Autopsy revealed a typical lepto-meningitis.

It can be said then, that rather large doses (2 cc.) of 1 per cent aqueous lysol solution introduced into the subarachnoid space of cats are well tolerated so far as can be judged by the clinical manifestations following the injections. The results of subarachnoid injections of this antiseptic, however, in the presence of meningitis produced with *B. lactis aerogenes*, have shown that the clinical course is not affected in any noticeable degree, and in no case was an infection actually prevented or overcome by a single injection of the chemical. The best therapeutic result was obtained when the antiseptic was preceded by an irrigation of the meninges with a bland solution (cf. Weed and Wegeforth (18)).

These findings agree in part with those obtained by Flexner and Amoss (6) in their experiments concerning the effect of lysol when introduced into the animal body. The investigations of these writers were directed toward establishing the superiority of serum over chemical treatment in the epidemic form of cerebro-spinal meningitis. That the curative power of the specific serum in the treatment of infections with the diplococcus intracellularis excels all other known remedies, cannot be questioned; the effect of chemicals on the reaction of the leucocytes toward the meningococcus constitutes, however, but one phase of the effect of such antiseptics in the central nervous system. The purulent non-meningococcic meningitides have as causative agents a variety of different organisms, none of which are as yet amenable to specific treatment. In these the mortality is extremely high, so that it may be assumed that the normal protective mechanisms of the individual as a whole are more or less powerless against the action of the bacteria. Under these conditions, the use of chemicals ideally is directed not only toward rallying these defensive agencies but also toward the direct destruction of the infecting organisms or the reduction of their virulence.

The experiments in this laboratory with subarachnoid injections of lysol indicate a fair degree of tolerance on the part of the animal for the chemical, but the inhibitory effect on the growth of the *B. lactis aerogenes* within the meninges has not been demonstrated. Examination of film preparations from the cerebro-spinal fluid and of sections, has not given evidence of any phagocytosis of the bacilli in the presence of lysol but it must be granted that under the experimental conditions in the cat phagocytosis of *B. lactis aerogenes* is only infrequently found in the meninges. The subarachnoid injection of lysol in the presence of meningeal infection with *B. lactis aerogenes* has not, in these experiments, prolonged the life of the animal; the subsequent pathological change in the meningeal spaces from injection of such antiseptics suggests the limitation of their employment.

Similar results have been obtained by subarachnoid injection of a tolerated dose of potassium permanganate when combined with a lethal subarachnoid inoculation of *B. lactis aerogenes*. A lumbar injection of 0.25 cc. of a 1-25,000 dilution of *B. lactis aerogenes* followed immediately by similar injections of 2 cc. of 1-1,000 potassium permanganate resulted in death from meningitis in twenty-eight hours. In this single experiment on a cat, there was no appreciable reduction of the virulence of the organism by the antiseptic, for the period of survival in this case was no longer than was usually encountered after similar dosages of the organisms employed. The dosage of the chemical in this instance was surely as large as could very well be employed, and the time of its injection, shortly after a direct inoculation, was favorable.

THE ISOLATION OF A PORTION OF THE SUBARACHNOID SPACE

The pathological study of the meninges of animals receiving intrathecal injections of antiseptics demonstrated the frequency with which the meningeal spaces became obliterated as a result of adhesions forming between the membranes. The presence of such an obstacle to the free circulation of the spinal fluid in the spinal canal was anticipated and for that reason the fluids taken

intra vitam from the animals for analytical purposes were obtained by occipito-atlantoid puncture. Ordinarily, a clear fluid may be obtained by lumbar puncture in a fairly large percentage of normal cats, but the procedure is quite unsatisfactory, as compared to the cistern puncture; even in the normal animal, a needle introduced into the lumbar canal will sometimes yield no fluid. The number of times that this occurs is sufficient to make a dry lumbar puncture of little value in determining the presence or absence of spinal fluid in this neighborhood. In several animals, which had been given lumbar subarachnoid injections of the various antiseptics, repeated efforts to obtain fluid by lumbar puncture were subsequently fruitless, but it could not be determined whether the failure to obtain fluid was due to faulty technique or whether adhesions had formed and blocked off the end of the subarachnoid space. The decision was made even more difficult by the fact that fluids were obtained in a few instances from the lumbar region but not nearly in as large a percentage of cases as normally. Furthermore, at autopsy in the fresh specimen or after microscopic examination, it is difficult, if adhesions are found, to say whether they were sufficient to produce a complete block of the subarachnoid space. Franca (7) assumed that the subarachnoid injection of lysol had effected complete cures, for his cases showed apparently no clinical evidences of residual effects, either of the meningeal infection or the antiseptics used for the cure. But the presence of anhydromyelia in one of this observer's cases and the frequent mention made by his follower, Glatard (9) of the difficulty of obtaining fluid by the lumbar route, late in the disease, after lysol injection, suggests that some obliteration of the subarachnoid space may also take place in the human subject following the use of this strong antiseptic.

To test the effectiveness of the experimental sero-fibrinous block of the subarachnoid channel following injection of the antiseptics, two methods were tried: injection of india ink through a lumbar needle at the time of the sacrifice of the animal, and inoculation of virulent organisms into the lumbar region for the purpose of ascertaining whether the infection would remain local-

ized to this area. In both methods, the secondary lumbar subarachnoid injection (ink or organisms) followed the initial lumbar subarachnoid injection of the corrosive antiseptic in five or six days, at a time when the cerebro-spinal fluid, obtained by cistern puncture had returned to normal. At that time, 2 cc. of the ink was injected into the lumbar subarachnoid space; in a normal animal, this amount, with the ordinary pressure of a syringe, should have caused a spread of the ink throughout the whole cord and base of the brain. In only one experiment was the lumbar region shut off from the rest of the subarachnoid space as demonstrated by the spread of ink. While the distribution of ink in all of the cases was patchy, the available channel for the flow of the ink in those cases which showed a persistence of circulation was restricted to a small part of the normal expanse of subarachnoid space, the interradicular zone being the area most frequently patent.

In two animals, after a preliminary subarachnoid injection of chloramine four and six days previously, a suitable lethal subarachnoid injection of *B. lactis aerogenes* was given into the lumbar needle. The first animal (cat 1452) died over night; the meninges were everywhere filled with organisms but showed very little evidence of a cellular reaction. The other (cat 1428) died in fifty hours; at autopsy the entire cord and brain were covered by a thick purulent meningeal exudate. The following protocol of cat 1428 is reproduced:

Cat 1428. Adult female

November 27, 1918. 3.05 p.m. Ether. Lumbar puncture, clear fluid. Subarachnoid injection 0.75 cc. 1 per cent chloramine. Immediately after injection, animal developed acute mania for several minutes.

November 28. Almost paralyzed in left hind leg, less so in right. Spinal fluid turbid, containing 3.5 grams of globulin per liter and 1400 white cells.

November 29. Cat is almost totally paralyzed in hind legs but right leg can just be used. Seems bright and active. Spinal fluid turbid, containing 14 grams of globulin per liter and 65 white cells.

November 30. Almost complete paraplegia; good general shape. Spinal fluid turbid, and contains 50 white cells per cubic millimeter.

December 2. Partial paraplegia still present. Bladder very much distended; emptied by pressure. Spinal fluid clear. Contains 0.1 gram globulin per liter and 10 white cells.

December 3. Condition of hind legs unchanged. General condition fair. 11.20 a.m. Ether, lumbar puncture: subarachnoid injection of 0.25 cc. (1-50,000) twenty-four hour meat infusion broth culture *B. lactis aerogenes*. 5.00 p.m. Practically total paraplegia. Retraction of head causes no reaction.

December 5. Still some movement in hind legs but not much. General condition good. Retraction of head causes no extensor out-thrust. 3.30 p.m. Dead in cage. Injected at once with 10 per cent formalin through aorta. Gross pathological diagnosis: massive acute leptomeningitis, generalized.

These two experiments, when considered with the results of the injection of india ink, indicate that the obliteration of the subarachnoid space, following local injections of these antiseptics, is usually incomplete. No apparent restriction of an infective process to the lumbar region was effected.

SUMMARY

The toxicity of certain antiseptics within the meninges has been tested and the results recorded in detail. Most of the chemical bodies employed possessed definite toxicity so that unless given in suitable dilution and amount, death of the animal would ensue. With chloramine and flavine, in addition to the initial toxicity, a secondary cause of death in five to ten days was brought about through direct injury to the central nervous system. With injection of small amounts of a suitable dilution the animals remain apparently normal but all have shown at autopsy pathological changes in the meninges.

The lesion consists of a more or less complete obliteration of the meningeal (subdural and subarachnoid) spaces with sero-fibrinous exudate; in the more severe cases the nervous system becomes involved in a process of destruction by direct continuity from the meninges. The blocking-off of the subarachnoid space

was complete in only one case, as demonstrated by the subsequent injection of ink. It was not, however, sufficient for the localization of the infection. The subarachnoid injection of lysol and potassium permanganate in the presence of an otherwise fatal meningeal infection, did not prolong the life of the animal.

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BENZYL ALCOHOL: ITS ANESTHETIC EFFICIENCY FOR MUCOUS MEMBRANES

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Macht (1) found that benzyl alcohol is anesthetic not only on injection, but also on application to mucous membranes. In view of the relative inefficiency of the cocain substitutes on mucosae, it appeared advisable to make comparative experiments on the frog skin and on the cornea, by the methods previously described, (2) and (3).

Two specimens of benzyl alcohol were tested: (M) a sample received from Dr. Macht, and diluted with 0.9 per cent NaCl shortly before use; and (X) commercial ampules, said to contain 2 per cent of the drug in normal saline, sent out by the manufacturers ten months previously, and opened just before use. Both specimens were neutral to litmus.

EFFICIENCY FOR FROG'S FOOT

The results¹ shown in table 1, place the threshold concentration at 0.125 per cent for specimen M and somewhat higher for specimen X. As concentration that is surely effective in ten minutes, one may accept 0.25 per cent.

This places the efficiency of benzyl alcohol fairly high, as shown in table 2.

EFFICIENCY FOR RABBIT'S CORNEA

A series of determinations were made by the method previously described, i.e., by applying the solutions with ten minute inter-

¹ Most of the determinations for the skin were made by G. E. Richardson.

vals, testing the anesthesia at the end of each period, and thus finding the number of applications necessary to secure anesthesia lasting at least ten minutes, and then the time necessary for recovery.

TABLE 1
Time required for complete sensory paralysis of frog skin by benzyl alcohol

BENZYL ALCOHOL	SPECIMEN M	SPECIMEN X
<i>per cent</i>		
1 and 2	Less than ten minutes	
0.5	{ Less than ten minutes Less than ten minutes	
0.25	{ Less than ten minutes	More than ten minutes Less than ten minutes Less than ten minutes
0.125	{ Ten x thirty minutes Less than ten minutes Thirty x forty minutes	More than ten minutes More than fifty minutes

TABLE 2
Comparative anesthetic efficiency of benzyl alcohol on frog skin

	LOWEST CONCENTRATION PARALYZING IN 10 MINUTES	EFFICIENCY RATIO COCAIN HYDROCHLORID = 1
	<i>per cent</i>	
Alpin hydrochlorid.....	0.125	1
Cocain hydrochlorid.....	0.125	1
Tropacocain hydrochlorid.....	0.25	$\frac{1}{2}$
Benzyl alcohol.....	0.25	$\frac{1}{2}$
Procain hydrochlorid.....	1.00	$\frac{1}{4}$

The results² as presented in table 3, show that specimen X is less than half as effective as specimen M. This speaks against the advisability of employing the agent in the form of ampules.

The comparative efficiency of the effective specimen with other local anesthetics is presented in table 4. This shows that benzyl alcohol compares quite favorably with other synthetic anesthetics.

² The determinations for the cornea were made by Miss J. R. Collacott.

As other experiments indicated that the action of benzyl alcohol is rather brief, a comparison was made between cocain and benzyl alcohol by testing the cornea at one-minute intervals after a single application. With both drugs, the anesthesia appeared

TABLE 3
Anesthetic efficiency of benzyl alcohol on rabbit's cornea

BENZYL ALCOHOL	SPECIMEN X		SPECIMEN M	
	A	R	A	R
<i>per cent</i>				
2.00	{	No anesthesia (2 experiments)	1	30
			1	10
1.00	{	No anesthesia	3*	10
			3*	10
0.5 and 0.25		No anesthesia	No anesthesia	

A = the number of applications, at ten minutes intervals, required to produce anesthesia lasting at least ten minutes. The results were considered negative, when four applications at ten minutes intervals did not abolish the reflex.

R = minutes elapsing between last application and complete recovery of sensation.

. *Almost complete.

TABLE 4
Comparative anesthetic efficiency of benzyl alcohol on the cornea

	LOWEST CONCENTRATION PRODUCING TEN MINUTES ANESTHESIA	EFFICIENCY RATIO
	<i>per cent</i>	
Cocain.....	0.5	1
Holocain.....	0.5	1
Beta eucain.....	1.0	$\frac{1}{2}$
Benzyl alcohol.....	1.0	$\frac{1}{2}$
Alpin.....	2.0	$\frac{1}{4}$
Procaïn.....	8.0	$\frac{1}{16}$

within one minute, if at all. The duration is shown in table 5. The solutions were made in 0.9 per cent sodium chloride.

The results indicate that the anesthetic threshold is about the same for the two drugs, and that the inferior showing of benzyl alcohol is due to the brief duration of its action. This can, however, be prolonged by increasing the concentration.

A more serious objection to the use of benzyl alcohol in the cornea is the fairly severe smarting. According to experiments on myself with 1 per cent solutions in 0.9 per cent sodium chloride, the smarting with 1 per cent benzyl alcohol is much greater than with 1 per cent cocain hydrochloride; eucaïn lactate, 1 per cent, being devoid of any irritation. The benzyl alcohol, however, is not intolerable, the smarting lasts only a few moments, soon ceding to complete anesthesia. The anesthesia was complete and about equally prompt with the three drugs, and lasted at least several minutes. The exact duration was not determined.

TABLE 5

Duration of anesthetic effect of cocain hydrochlorid and of benzyl alcohol on rabbit's cornea, after a single application

PERCENTAGE	DURATION OF ANESTHESIA	
	COCAIN	BENZYL ALCOHOL
	minutes	minutes
2.00		10 x 15, 20 x 30
1.00		6
0.5	21	5
0.25	12	3
0.125	Ineffective	Ineffective

EFFICIENCY ON GUMS

Method. The best results are obtained by saturating a pledget of cotton with the solution, and placing it between the gums and lips, opposite the second incisor. The sensation is tested by touching with the point of a pencil, at one or two minute intervals. There is a somewhat sharp sensation or taste, followed within one minute by numbness. This is not increased by repeating the applications. Normal sensation returns rapidly after the cotton is removed; even with the concentrated alcohol, this does not take longer than three minutes.

Results. With the undiluted alcohol, anesthesia was complete within one minute. After removal of the cotton, the anesthesia had weakened to numbness in one and one-half minutes, and sensation was almost normal in three minutes.

With 2 per cent and 1 per cent solutions, anesthesia was incomplete. One-half per cent solutions were practically ineffective; 1 per cent may therefore be considered the threshold concentrations.

The X solutions were less effective than the M sample, as seen most plainly with the 1 per cent solutions: The M sample gave definite though incomplete anesthesia; the X sample only very slight numbness.

ANESTHESIA OF THE TONGUE

Five cubic centimeters of a 1 per cent solution were held in the mouth for one minute. This produced at once a prickly sensation somewhat similar to aconite; very slight anesthesia and increased flow of saliva. Five minutes later, the tongue feels rather rough, but one could scarcely describe the effect as anesthesia.

The experiment was repeated, holding the solution in the mouth for ten minutes. The effects were the same. The prickly sensation could still be felt, though much weakened, after an hour. The taste was noticeable for about the same time.

It appears that 1 per cent benzyl alcohol does not produce satisfactory anesthesia of the tongue, even after ten minutes contact. It causes a prickling sensation which fades slowly. The taste is also quite persistent.

EFFICIENCY IN THE NOSTRILS

A pledget of cotton dipped into the undiluted benzyl alcohol and inserted into the nostrils is painless and produces distinct but incomplete anesthesia. Presumably, the anesthesia would be more efficient in the deeper portions of the nose, where the epithelium is more absorbent.

Two per cent solution, applied on cotton, produces very incomplete anesthesia.

EFFICIENCY ON THE INTACT SKIN

Undiluted benzyl alcohol was painted freely on the dorsum of the hand, between the thumb and forefinger. This produced at

once a very slight dulling in sensibility, but so slight that touching with cotton was felt practically as easily as on the other hand. The anesthesia did not deepen with time.

CONCLUSIONS

Benzyl alcohol is a fairly efficient anesthetic for intact mucous membranes, greatly surpassing procain; ranking about with alypin, betaeucain; and somewhat weaker than holocain or cocain. Its action is not as lasting as that of cocain, and even 1 per cent solutions produce considerable smarting.

Commercial solutions in ampules appear to deteriorate somewhat, so that it is preferable to use freshly made solutions, when possible.

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THE ACTION OF DRUGS ON THE OUTPUT OF EPINEPHRIN FROM THE ADRENALS

IV. STROPHANTHIN

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INTRODUCTION

The statement occasionally met with in the literature, that strophanthin causes a decided increase in the rate of liberation of epinephrin from the adrenals, has no real experimental basis. Gley (1) mentions strophanthin along with nicotine and anagyrin in speaking of the mode of action of drugs which markedly increase the blood pressure after the bulbospinal centers have been eliminated. But while he cites experiments which he interprets, erroneously we believe (2), as indicating that practically the whole (peripheral) effect of nicotine (and anagyrin) is due to an augmenting influence upon the epinephrin output, he does not state that he made any observations upon strophanthin.

Richards and Wood (3) came to the conclusion that "the intravenous injection of strophanthin is regularly followed by the development in the blood of the capacity to cause decrease of tonus and inhibition of contractions in an isolated strip of intestinal muscle in a manner indistinguishable from that possessed by epinephrine." They obviously consider that their experiments demonstrate a markedly increased rate of epinephrin output under the influence of strophanthin. In reality, however, they show at most that the concentration of epinephrin in blood collected from the inferior vena cava above the level of the

adrenals after strophanthin is greater than before strophanthin. The method used by them (collection of cava blood by a catheter) did not permit the estimation of changes in the rate of the blood flow, and it is clear that if the blood flow was diminished at the time a sample was collected, the concentration must necessarily have been increased, even if no change whatever had occurred in the rate of epinephrin output. It is certain that the blood flow in the cava must often have been diminished at the time the samples after strophanthin injection were collected. Indeed, it is stated that samples were sometimes taken when the animal was dying and in one case a sample was taken after the heart had stopped. Such specimens of blood would necessarily contain epinephrin in a greater concentration than specimens collected with a good flow, without the administration of any drug. No quantitative estimations of the epinephrin output before and after the injection of strophanthin were made, nor was it possible to make them with the method employed. It ought to be explained that these observers were fully aware that the method was defective and only adopted it, so to say, under protest. They point out "that this method is highly faulty in that the blood from the suprarenals is diluted with that from all of the structures whose veins enter the cava below the suprarenal." This is a serious fault, but the really fatal objection is that no provision is made for the measurement of the rate of blood flow. Even with samples collected while the arterial pressure was raised by strophanthin, it is impossible to assume that the blood flow in the upper part of the cava was not reduced. For the vasoconstriction on which the increase of arterial pressure mainly depends may be expected to diminish the rate of the venous flow.

Our experiments were made principally on cats because we have now a large statistical material from which to calculate the normal epinephrin output in this animal, under given experimental conditions, without the administration of any drug except the ordinary anesthetics. It is, therefore, easy to compare in any case an epinephrin output observed after a drug, with the average normal output. Of course comparison of the

output before and after the administration of the drug was made in each experiment and this is the essential thing. But it is often of value to know in addition whether a given result lies within the normal range or clearly surpasses the normal maximum, or falls decidedly below the normal minimum.

Technique. The technique has already been described sufficiently in previous papers. The cats were anesthetized with ether, except in two cases, where a blood pressure assay was to be made, when to ensure a steadier blood pressure curve urethane was employed. In one dog the cerebral peduncles were divided under ether and thereafter no anesthetic was given. But for reasons which will be given more fully in a succeeding paper, although some of them may be indicated in discussing this experiment, we consider that as a rule such mutilations of the central nervous system introduce serious complications in work on the epinephrin output. To practice them for the purpose of avoiding a supposed action of anesthetics upon the output, is, we believe, in general to choose the greater in preference to a lesser, if not an imaginary evil.

As in our previous work, we relied chiefly upon experiments in which blood was collected directly from the adrenals by cannulae in a pocket of the inferior cava. The blood specimens were at once placed on ice, and assayed on rabbit intestine (and uterus) segments. Corroborative evidence was sought by blood pressure auto-assays (collection of adrenal blood in a cava pocket for a given time with estimation of the amount of epinephrin from the blood pressure reaction when the pocket was opened).

The strophanthin (labelled Merck, U. S. P., ix), made up in salt solution to such a concentration that about 0.5 cc. corresponded to the dose to be given was injected into the jugular vein and washed in with 2 cc. of salt solution. It was usually put in more rapidly than in the experiments of Richards and Wood, as our experience with nicotine (2) showed that a transient, though relatively intense stimulation of the epinephrin output by that drug was completely missed when collection of the adrenal blood was delayed beyond half a minute to a minute after the beginning of the injection. We were not generally able to use as large doses as Richards and Wood without killing the animal before satisfactory adrenal blood samples could be procured. This was only partly due to the more rapid injection. For in 5 experiments in which the drug was injected slowly one cat died almost

immediately after the gradual administration of 0.07 mgm. per kilogram during 10 minutes, another after the administration of 0.046 mgm. per kilogram during 10 minutes. The other three cats received respectively 0.05 mgm. per kilogram during $12\frac{1}{2}$ minutes, 0.043 mgm. per kilogram during $12\frac{1}{2}$ minutes, and 0.045 mgm. per kilogram during $12\frac{1}{2}$ minutes. The last cat was under urethane, the others under ether. The blood pressure, which in all cases was somewhat increased during the injection of the strophanthin, remained at a satisfactory level in these three animals while the specimens of adrenal blood were being collected. It was demonstrated by an additional small dose at the end of the experiment that the doses given originally were as great as could possibly be employed. Since in forming the cava pocket in our experiments, the abdominal aorta was always tied near the bifurcation, the nominal dose per kilogram of animal may be somewhat less than the real dose. The point is of no importance in itself and we have entered into these details merely to make it clear that the results which we obtained were not due to the employment of doses below the minimum effective dose. We are certain that the doses were pushed to the utmost limit consistent with the successful carrying out of the observations.

The results of the experiments in which a relatively large dose was injected slowly did not differ noticeably from those of the other experiments, unless, indeed, in being more consistently negative as regards any demonstrable effect of the drug upon the epinephrin output.

EXPERIMENTS WITH DIRECT COLLECTION OF ADRENAL BLOOD AND EPINEPHRIN ASSAY ON RABBIT SEGMENTS

In the first experiment to be quoted, the dose was intermediate between the largest and smallest doses employed in the investigation (0.125 mgm. in a 3.86 kgm. cat). The result was negative. No change in the epinephrin output, beyond the limits of error inherent in the method, could be made out.

Condensed protocol. Cat 293; male; weight, 3.865 kgm.

Anesthetized with ether. Obtained specimen of indifferent blood from jugular. Cut vagi. Made cava pocket. Collected adrenal blood.

- 11.01½ a.m. First specimen, 3.4 grams in 30 seconds (6.8 grams per minute).
- 11.02 a.m. Second specimen, 8.8 grams in 90 seconds (5.87 grams per minute). Blood pressure at end of collection of second specimen was 134 mm. of mercury (fig. 1, observation 4).
- 11.09½ a.m. Injected intravenously 0.125 mgm. strophanthin (fig. 1, observations 5 to 6).
- 11.10½ a.m. Third adrenal specimen, 7.5 grams in 60 seconds (7.5 grams per minute).
- 11.11½ a.m. Fourth adrenal specimen, 8.6 grams in 90 seconds (5.7 grams per minute). Blood pressure at beginning of collection of third specimen was 186 mm. of mercury (fig. 1, observation 7); at beginning of collection of fourth specimen, 182 mm. (observation 8); at end of collection of fourth specimen 144 mm. (observation 9).
- 11.25 a.m. Fifth adrenal specimen, 2.35 grams in 30 seconds (4.7 grams per minute).
- 11.25½ a.m. Sixth adrenal specimen, 7.05 grams in 150 seconds (2.8 grams per minute). Blood pressure at beginning of collection of fifth specimen was 130 mm. of mercury (fig. 1, observation 10); at beginning of collection of sixth specimen 117 mm. (observation 11); at end of collection of sixth specimen 74 mm. (observation 12). Obtained another specimen of venous blood. Combined weight of adrenals 0.377 gram.

Figure 1 indicates the points on the blood pressure curve at which the various adrenal specimens were collected. A few samples of the tracings used for the epinephrin assay are reproduced in figures 2 to 4. The tracings are far too numerous to permit the reproduction of the assay of even one experiment completely. The second specimen, collected before administration of strophanthin, was found to be weaker than 1:6,660,000 adrenalin (observations not reproduced), weaker than 1:8,000,000, stronger than 1:12,500,000 (not reproduced), stronger than 1:10,000,000 (fig. 2). It was finally taken at 1:9,000,000, corresponding to an output of epinephrin of 0.00065 mgm. per minute for the cat, or 0.00017 mgm. per kilogram per minute.

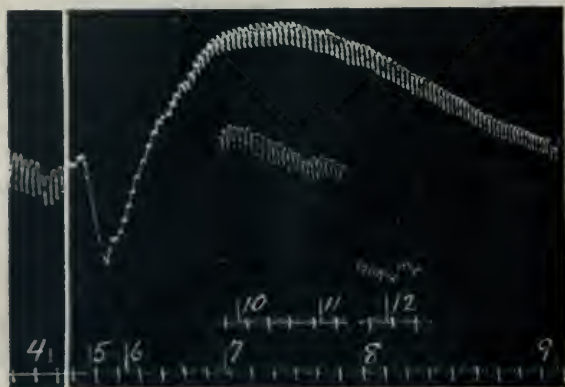


FIG. 1. BLOOD PRESSURE TRACING. CAT 293

4, The end of collection of the second adrenal specimen; 5 to 6, the intravenous injection of strophanthin; 7, the beginning of collection of the third adrenal specimen; 8, beginning of collection of the fourth adrenal specimen; 9, end of collection of the fourth adrenal specimen; 10, beginning of collection of the fifth adrenal specimen; 11, beginning of collection of the sixth adrenal specimen; 12, end of collection of the sixth adrenal specimen. Line of zero pressure corresponds with time trace and is moved up 28 mm. and the figure then reduced to two-thirds. As in all the other blood pressure tracings except when otherwise mentioned, time is marked in seconds and ten seconds.

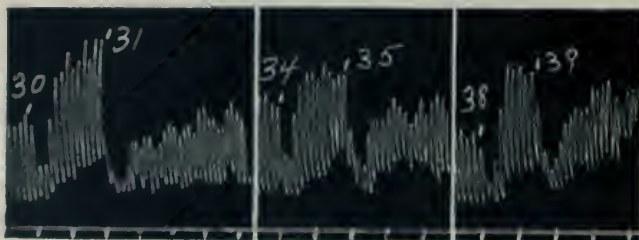


FIG. 2. INTESTINE TRACINGS. BLOODS FROM CAT 293

At 30, 34, and 38, Ringer was replaced by jugular blood and this at 31 by jugular blood to which was added adrenalin to make a concentration of 1:8,000,000; at 35 by the second adrenal specimen (collected before injection of strophanthin); at 39 by jugular blood to which was added adrenalin to make a concentration of 1:10,000,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). In all of the intestine and uterus tracings the time is marked in half minutes. Reduced to three-fifths.

The third and fourth specimens were found to be decidedly stronger than 1:10,000,000, but weaker than 1:8,000,000 (fig. 3). The third specimen was found to be much weaker than 1:6,660,000, weaker than 1:8,300,000, and somewhat stronger than 1:9,150,000. The fourth specimen was somewhat stronger than the third (confirmed by other observations), and approxi-

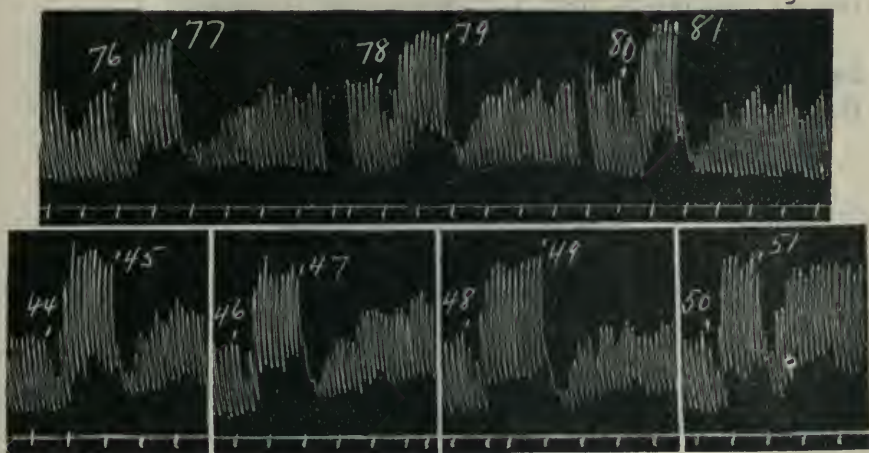


FIG. 3. INTESTINE TRACINGS. BLOODS FROM CAT 293

At 44, 46, 48, 50, 76, 78, and 80, Ringer was replaced by venous blood (collected after the injection of strophanthin); and this at 45 by the third adrenal specimen (collected immediately after the injection of strophanthin); at 47 by the fourth adrenal specimen (collected two minutes after injection of strophanthin); at 49 by venous blood to which was added adrenalin to make a concentration of 1:8,000,000; at 51 by venous blood to which was added adrenalin to make a concentration of 1:10,000,000; at 77 by venous blood to which was added adrenalin to make a concentration of 1:4,100,000; at 79 by venous blood to which was added adrenalin to make a concentration of 1:5,000,000; and at 81 by the sixth adrenal specimen (collected sixteen minutes after injection of strophanthin). All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to three-fifths.

mately equal to 1:8,300,000 (observations not reproduced). It was confirmed by uterus tracings that the second, third, and fourth specimens were not very different in strength, the fourth being slightly the strongest (fig. 4). Indifferent blood in the same dilution gave only a small increase of tone.

Taking the third specimen (collected one to two minutes after strophanthin injection) at 1:9,000,000, we get for the output of epinephrin 0.0008 mgm. per minute for the cat, or 0.0002 mgm. per kilogram per minute. Taking the fourth specimen (collected two to three and a half minutes after the strophanthin injection) at 1:8,300,000, we get 0.0007 mgm. per minute for the cat, or 0.00018 mgm. per kilogram per minute.

The sixth adrenal specimen, collected sixteen to eighteen and a half minutes after the strophanthin injection, was much stronger than the other specimens, corresponding to the much slower



FIG. 4. UTERUS TRACINGS. BLOODS FROM CAT 293

At 87 Ringer was replaced by the fourth adrenal specimen (collected two minutes after injection of strophanthin); at 88 by the third adrenal specimen (collected immediately after injection of strophanthin); at 89 by the second adrenal specimen (collected before injection of strophanthin). All the bloods were diluted with two volumes Ringer. Reduced to one-half.

blood flow. It was found to be stronger than 1:5,000,000 (fig. 3, observations 79 and 81, confirmed by another pair of observations not reproduced), decidedly weaker than 1:3,300,000 (2 pairs of observations, not reproduced), not very different from 1:4,100,000 (fig. 3, observations 77 and 81). Taking the sixth specimen at 1:4,000,000, we get an epinephrin output of 0.0007 mgm. per minute for the cat, or 0.00017 mgm. per kilogram per minute.

In the next experiment (cat 295) the dose of strophanthin, calculated on the body weight, was two and a half times as

great. The result was the same, no increase in the epinephrin output, beyond the limits of error, was found in an adrenal blood specimen collected two to four minutes after administration of the strophanthin. A more remote specimen could not be obtained as the animal died from the effects of the drug.

Condensed protocol. Cat 295; female; weight, 2.9 kgm.

Anesthetized with ether. Obtained specimen of indifferent blood from jugular. Cut vagi. Made cava pocket. Collected adrenal blood.

- 10.59 a.m. First specimen, 2.2 grams in 30 seconds (4.4 grams per minute).
- 10.59½ a.m. Second specimen, 6.45 grams in 120 seconds (3.2 grams per minute). Blood pressure at end of collection of second specimen was 98 mm. of mercury (fig. 5, observation 4).
- 11.07 a.m. Injected intravenously 0.24 mgm. strophanthin (fig. 5, observations 5 to 6).
- 11.08 a.m. Third adrenal specimen, 5.05 grams in 60 seconds (5.05 grams per minute).
- 11.09 a.m. Fourth adrenal specimen, 5.45 grams in 120 seconds (2.75 grams per minute). Blood pressure at beginning of collection of third specimen was 140 mm. of mercury (fig. 5, observation 7); at beginning of collection of fourth specimen 130 mm. (observation 8); at the end of collection of the fourth specimen 66 mm.

Obtained another specimen of venous blood. Combined weight of adrenals 0.372 gram.

The points on the blood pressure curve at which the adrenal blood samples were procured are given in figure 5. The second specimen, collected before the administration of strophanthin, was shown to be weaker than 1:6,660,000 adrenalin, not far from 1:8,300,000, stronger than 1:10,000,000. It was taken at 1:8,000,000, corresponding to an output of 0.0004 mgm. per minute for the cat, or 0.00014 mgm. per kilogram per minute.

The fourth specimen (beginning two minutes, or allowing for the dead space in cannula and cava, about one and three-quarter



FIG. 5. BLOOD PRESSURE TRACING. CAT 295

4, The end of collection of the second adrenal specimen; 5 to 6, injection of strophanthin; 7, beginning of collection of the third adrenal specimen; 8, beginning of collection of the fourth adrenal specimen. Line of zero pressure corresponds with the time trace and is moved up 20 mm. and the figure then reduced to two-thirds.



FIG. 6. BLOOD PRESSURE TRACING. CAT 290

4, The end of collection of the second adrenal specimen; 5, injection of strophanthin; 6, beginning of collection of the third specimen; 7, beginning of collection of the fourth specimen; 8, started artificial respiration near the end of collection of fourth specimen. Line of zero pressure corresponds with time trace. Reduced to two-thirds.

minutes after the injection of strophanthin), was shown to be stronger than 1:6,660,000 adrenalin, much weaker than 1:1,660,000, weaker than 1:3,300,000, and not much different from 1:5,000,000. Taking it at 1:5,000,000, we get an output of 0.00055 mgm. per minute for the cat, or 0.00019 mgm. per kilogram per minute.

In the next experiment (cat 290) the largest dose used in the series was given (0.22 mgm. per kilogram). The animal died within three minutes of injection of the strophanthin. The points on the blood pressure curve at which the adrenal blood specimens were procured are indicated in figure 6. The assay revealed no certain increase in epinephrin output in the samples collected after strophanthin, as compared with the original output.

Condensed protocol. Cat 290; female; weight, 2.265 kgm.

Anesthetized with ether. Obtained specimen of indifferent blood from jugular. Cut vagi. Made cava pocket. Collected adrenal blood.

- 11.47½ a.m. First specimen, 0.7 gram in 30 seconds (1.4 grams per minute).
- 11.48 a.m. Second specimen, 3.3 grams in 180 seconds (1.1 grams per minute). Blood pressure at end of collection of second adrenal specimen was 98 mm. of mercury (fig. 6, observation 4).
- 11.56½ a.m. Injected intravenously 0.5 mgm. strophanthin (fig. 6, observation 5).
- 11.57¾ a.m. Third adrenal specimen, 0.65 gram in 30 seconds (1.3 grams per minute).
- 11.58¼ a.m. Fourth adrenal specimen, 0.6 gm. in 90 seconds (0.4 gram per minute). Blood pressure at beginning of collection of third specimen was 156 mm. of mercury (fig. 6, observation 6); at beginning of collection of fourth specimen 135 mm. (observation 7); near the end of collection of fourth specimen, artificial respiration was started (observation 8); blood pressure was 20 mm. The cat was dead at the end of collection of the fourth specimen.

Combined weight of adrenals 0.296 gram.

The second adrenal specimen, taken before strophanthin, was much stronger than 1:4,000,000, stronger than 1:2,750,000 (fig. 7, observations 4, 6 and 8), perhaps somewhat weaker than 1:2,000,000 (fig. 7, observations 8 and 10), but about the same as 1:2,000,000 in another pair of observations (not reproduced). Taking the second specimen at 1:2,000,000, we get 0.00055 mgm. as the output of epinephrin per minute for the cat, or 0.00024 mgm. per kilogram per minute.

The collection of the fourth adrenal specimen was begun one and three-quarter minutes after the injection of strophanthin.



FIG. 7. INTESTINE TRACINGS. BLOODS FROM CAT 290

At 3, 5, 7 and 9, Ringer was replaced by jugular blood and this at 4 by jugular blood to which was added adrenalin to make a concentration of 1:4,000,000; at 6 by jugular blood to which was added adrenalin to make a concentration of 1:2,750,000; at 8 by jugular blood to which was added adrenalin to make a concentration of 1:2,000,000; at 10 by the second adrenal specimen (collected before injection of strophanthin). All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

Allowing for the dead space, this would correspond approximately to the point at which the blood pressure was attaining its maximum. The concentration of epinephrin in the fourth specimen was found to be more than 1:1,300,000, less than 1:700,000, and somewhat less than 1:1,000,000. Taking it at 1:1,100,000, we get 0.00035 mgm. per minute as the epinephrin output for the cat, or 0.00016 mgm. per kilogram per minute. Allowing for the fact that during the last half minute of the nominal period of collection of this specimen the blood pressure was falling most abruptly and that the blood flow had almost

ceased, this is practically the same output as before the drug was administered. The proportion of serum in the blood was 57 per cent as determined by the electrical method, and 56 per cent as determined by the haematocrite (after twenty minutes rotation). The concentration of epinephrin in the serum of the fourth specimen was accordingly about 1:630,000, not much less than the possible normal maximum. Unless strophanthin has the power of causing the epinephrin concentration to rise beyond the possible normal maximum, of which we have seen no clear evidence, the calculated output of epinephrin in this specimen could hardly be expected to show an increase. The experiment is cited mainly to illustrate the point that observations which purport to demonstrate an increased output of epinephrin under the influence of strophanthin merely because after injection of the drug an increased concentration of epinephrin may be found in the blood of the inferior cava, in the absence of information as to changes in the rate of the blood flow, cannot possibly prove what they profess to prove. That epinephrin may be more readily detected in the cava blood at the adrenal level when an animal is dying or even after the heart has stopped than when a good circulation is being maintained is perfectly true, but this has nothing to do with any stimulating action of strophanthin upon the output.

In the next experiment (cat 312) the effect of a much smaller dose (0.0018 mgm. per kilogram) was investigated. This was the smallest dose employed in the series, but it was sufficient to produce a distinct effect upon the blood pressure, a rise succeeded by a progressive fall. The animal was still in good condition when the last adrenal blood sample was procured.

Condensed protocol. Cat 312; female; weight, 2.8 kgm.

Anesthetized with ether. Obtained specimen of indifferent blood from jugular. Cut vagi. Made cava pocket. Collected adrenal blood.

10.22 a.m. First specimen, 1.15 grams in 30 seconds (2.3 grams per minute).

- 10.22½ a.m. Second specimen, 4.3 grams in 180 seconds (1.43 grams per minute). Blood pressure at end of collection of second specimen was 54 mm. of mercury (fig. 8, observation 3).
- 10.29¼ a.m. Injected intravenously 0.05 mgm. strophanthin (fig. 8, observations 4 to 5).
- 10.30 a.m. Third adrenal specimen, 4.15 grams in 60 seconds (4.15 grams per minute).
- 10.31 a.m. Fourth adrenal specimen, 4.95 grams in 120 seconds (2.5 grams per minute).
- 10.33 a.m. Fifth adrenal specimen, 4.1 grams in 180 seconds (1.4 grams per minute). Blood pressure at beginning of collection of third adrenal specimen was 116 mm. of mercury (fig. 8, observation 6); at beginning of collection of fourth specimen 106 mm. (observation 7); at beginning of collection of fifth specimen 74 mm. (observation 8); at end of collection of fifth specimen 52 mm. (observation 9).
- 10.58 a.m. Sixth adrenal specimen, 1.1 grams in 30 seconds (2.2 grams per minute).
- 10.58½ a.m. Seventh adrenal specimen, 5.15 grams in 240 seconds (1.3 grams per minute). Blood pressure at beginning of collection of sixth adrenal specimen was 76 mm. of mercury (fig. 8, observation 10); at beginning of collection of seventh specimen 70 mm. (observation 11); at end of collection of seventh specimen 48 mm. (observation 12).

Obtained another specimen of venous blood. Combined weight of adrenals 0.34 gram.

Figure 8 shows the points on the blood pressure curve at which the adrenal bloods were collected. No evidence was obtained of any increase in the rate of output, with the exception of a possible small increase in the last specimen, collected half an hour after injection of the strophanthin. The initial output, however, before strophanthin was below the average normal output in cats anesthetized by ether, and the output calculated for the last specimen did not at all exceed the normal average. On the other hand, there was a distinct diminution in the output for

the specimen (fourth), collected about two minutes after the administration of the drug. The assay was such that there could be no doubt that at this time the output was depressed for a brief period. This is about the only instance in which such a depression has been noted in this investigation, and we do not know whether it should be attributed to the strophanthin or not. Where an abrupt change in the rate of blood flow is coincident with the collection of a sample, it is obvious that the blood already in the adrenals with the lower concentration of epinephrin corresponding to a greater blood flow will pass into a

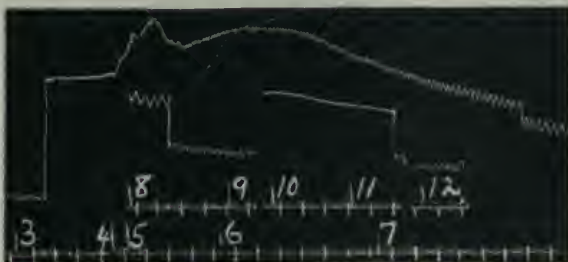


FIG. 8. BLOOD PRESSURE TRACING. CAT 312

3, The end of collection of the second adrenal specimen; 4 to 5, intravenous injection of strophanthin; 6, beginning of collection of the third adrenal specimen; 7, beginning of collection of fourth adrenal specimen; 8, beginning of collection of fifth adrenal specimen; 9, end of collection of fifth adrenal specimen; 10, beginning of collection of sixth adrenal specimen; 11, beginning of collection of seventh adrenal specimen; 12, end of collection of seventh adrenal specimen. Line of zero pressure corresponds with time trace and is moved up 15 mm.

sample collected with a lower average blood flow and will, to some extent, diminish its concentration. Considering, however, the maximum possible amount of blood in the adrenal medulla and the blood flow when the third and fourth specimens were obtained in this experiment, this factor could only play an insignificant part.

A few samples of the tracings used in the epinephrin assay are reproduced in figures 9 to 11. The second specimen, collected before injection of strophanthin, was weaker than 1:3,000,000 adrenalin, stronger than 1:6,000,000 (fig. 9). In other obser-

vations (not reproduced) it was shown that the second specimen was nearer 1:3,000,000 than 1:6,000,000, and not far from 1:4,000,000, corresponding to an output of epinephrin of 0.00035 mgm. per minute for the cat, or about 0.00013 mgm. per kilogram per minute.

The third specimen, collected forty-five seconds, or allowing for the dead space, not more than thirty-five seconds after the injection of strophanthin, was much weaker than 1:3,000,000,

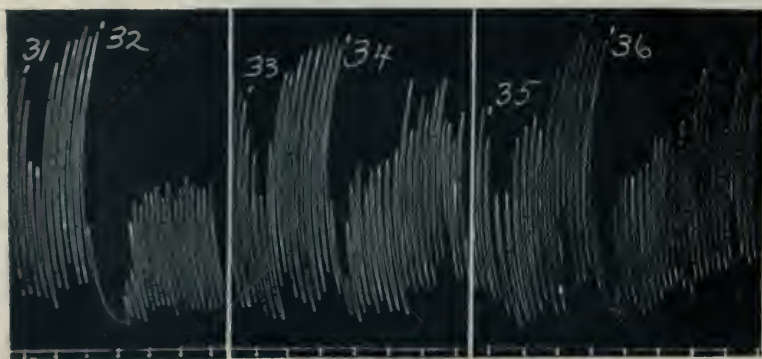


FIG. 9. INTESTINE TRACINGS. BLOODS FROM CAT 312

At 31, 33 and 35 Tyrode's solution was replaced by jugular blood and this at 32 by jugular blood to which was added adrenalin to make a concentration of 1:3,000,000; at 34 by jugular blood to which was added adrenalin to make a concentration of 1:6,000,000; at 36 by the second adrenal specimen (collected before injection of strophanthin). All the bloods were diluted with three volumes Tyrode's solution (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

weaker than 1:6,000,000, somewhat weaker than 1:9,000,000, not far from 1:12,000,000 (fig. 10, observations 62 and 64). Taking it at 1:12,000,000, we get an output of 0.00035 mgm. per minute for the cat, the same as for the second specimen.

The fourth specimen, obtained one and three-quarter minutes, or allowing for the dead space, one and a half minutes after the administration of the drug, was found to be much weaker than the second, and also decidedly weaker than the third specimen (fig. 10, observations 64 and 70). It was much weaker than

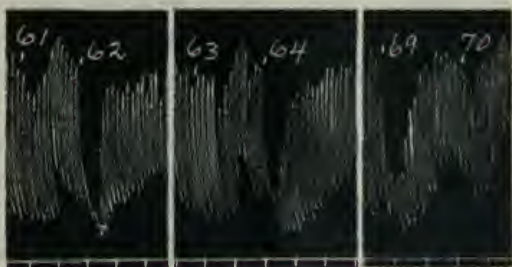


FIG. 10. INTESTINE TRACINGS. BLOODS FROM CAT 312

At 61, 63, and 69, Tyrode's solution was replaced by venous blood and this at 62 by venous blood to which was added adrenalin to make a concentration of 1:12,000,000; at 64 by the third adrenal specimen (collected immediately after injection of strophanthin); at 70 by the fourth adrenal specimen (collected two minutes after injection of strophanthin). All the bloods were diluted with three volumes Tyrode's solution (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

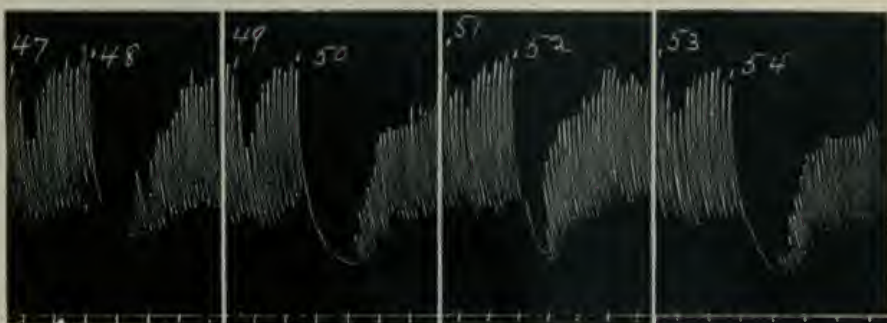


FIG. 11. INTESTINE TRACINGS. BLOODS FROM CAT 312

At 47, 49, 51, and 53 Tyrode's solution was replaced by venous blood and this at 48 by the fifth adrenal specimen (collected four minutes after injection of strophanthin); at 50 by venous blood to which was added adrenalin to make a concentration of 1:2,250,000; at 52 by venous blood to which was added adrenalin to make a concentration of 1:3,750,000, at 54 by the seventh adrenal specimen (collected thirty minutes after injection of strophanthin). All the bloods were diluted with three volumes Tyrode's solution (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

1:9,000,000 adrenalin (shown by 2 separate pairs of observations), and probably weaker than 1:18,000,000, corresponding to an output of not more than 0.00014 mgm. per minute for the cat, or 0.00005 mgm. per kilogram per minute.

The fifth adrenal specimen, collected from the fourth to the seventh minute after strophanthin, was shown to be much stronger than the third specimen, weaker than 1:2,250,000 adrenalin (fig. 11, observations 48 and 50), somewhat stronger than 1:3,750,000 (fig. 11, observations 48 and 52). It was taken at 1:3,500,000, corresponding to an output of 0.0004 mgm. per minute for the cat, or 0.00014 mgm. per kilogram per minute. Up to this point then no increase had occurred in the epinephrin output.

The seventh specimen, procured half an hour after injection of strophanthin, was decidedly stronger than 1:3,750,000 adrenalin (fig. 11, observations 52 and 54) and than the fifth specimen, not far from 1:2,250,000 (fig. 11, observations 50 and 54), corresponding to an output of 0.00055 mgm. per minute for the cat, or 0.0002 mgm. per kilogram per minute.

The next experiment (cat 294) is cited because here too there appeared to be a somewhat increased output, although not beyond the normal range, in a remote adrenal blood specimen (collected twenty minutes after strophanthin injection), whereas the specimens collected within a few minutes of the administration of the drug showed no increase.

Condensed protocol. Cat 294; male; weight, 2.73 kgm.

Anesthetized with ether. Obtained specimen of indifferent blood from jugular. Cut vagi. Made cava pocket. Collected adrenal blood.

- 11.42½ a.m. First specimen, 2.65 grams in 30 seconds (5.3 grams per minute).
11.43 a.m. Second specimen, 6.85 grams in 90 seconds (4.8 grams per minute). Blood pressure at end of collection of second adrenal specimen was 109 mm. of mercury (fig. 12, observation 4).
11.50 a.m. Injected intravenously 0.15 mgm. strophanthin (fig. 12, observations 5 to 6).

- 11.51 $\frac{3}{4}$ a.m. Third adrenal specimen, 5.3 grams in 60 seconds (5.3 grams per minute).
- 11.52 $\frac{3}{4}$ a.m. Fourth adrenal specimen, 6.2 grams in 120 seconds (3.1 grams per minute). Blood pressure at beginning of collection of third specimen was 142 mm. of mercury (fig. 12, observation 7); at beginning of collection of 4th specimen 124 mm. (observation 8); at end of collection of fourth specimen 80 mm. (observation 9).
- 12.09 p.m. Fifth adrenal specimen, 1.3 grams in 30 seconds (2.6 grams per minute).

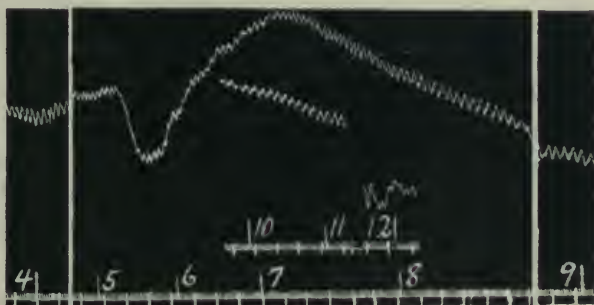


FIG. 12. BLOOD PRESSURE TRACING. CAT 294

4, The end of collection of the second adrenal specimen; 5 to 6, intravenous injection of strophanthin; 7, beginning of collection of third adrenal specimen; 8, beginning of collection of fourth adrenal specimen; 9, end of collection of fourth adrenal specimen; 10, beginning of collection of fifth adrenal specimen; 11, beginning of collection of sixth adrenal specimen; 12, end of collection of sixth adrenal specimen. Line of zero pressure corresponds with time trace and is moved up 18 mm. and the figure then reduced to two-thirds.

- 12.09 $\frac{1}{2}$ p.m. Sixth adrenal specimen, 4.5 grams in 180 seconds (1.5 grams per minute). Blood pressure at beginning of collection of fifth adrenal specimen was 98 mm. of mercury (fig. 12, observation 10); at beginning of collection of sixth specimen 78 mm. (observation 11); at end of collection of sixth specimen 60 mm. (observation 12).

Obtained another specimen of venous blood. Combined weight of adrenals 0.316 gram.

The points on the blood pressure curve at which the adrenal specimens were procured are indicated in figure 12.

The assay showed that the second specimen, collected before strophanthin, was stronger than 1:8,300,000 adrenalin, weaker than 1:5,000,000, weaker than 1:6,000,000. It was taken at 1:7,500,000, equivalent to an epinephrin output of 0.00065 mgm. per minute for the cat, or 0.00024 mgm. per kilogram per minute.

The third specimen, obtained about a minute after the end of the strophanthin injection, was weaker than 1:6,660,000, somewhat weaker than 1:8,300,000, somewhat stronger than 1:10,000,000. It was taken at 1:9,000,000, corresponding to an output of 0.006 mgm. per minute for the cat, or 0.00022 mgm. per kilogram per minute.

The fourth specimen, collected from two to four minutes after the end of the strophanthin injection, was found to be weaker than 1:5,000,000 adrenalin, probably slightly weaker than 1:6,000,000. It was taken at 1:6,200,000, equivalent to an output of 0.0005 mgm. per minute for the cat, or 0.0018 mgm. per kilogram per minute.

The sixth specimen, collected nineteen to twenty-two minutes after the administration of the drug, when the blood flow was much less than in the case of the other specimens, although still fair, was found to be stronger than 1:1,660,000 adrenalin, slightly stronger than 1:1,250,000, weaker than 1:830,000. It was confirmed by uterus tracings that the sixth specimen was much stronger than the third and fourth. Taking its concentration at 1:1,200,000, we get an output of 0.00125 mgm. per minute for the cat, or 0.00045 mgm. per kilogram per minute, nearly double the output at the time of collection of the second specimen, although not beyond the maximum output observed in anesthetized cats. We believe it would be unwarrantable to conclude that this small and remote effect was occasioned by a direct excitation of the secretion by strophanthin, seeing that in the earlier specimens at the time the drug was causing a decided vasoconstrictor stimulation, there was no increase whatever. It seems likely that a small and inconstant increase in the rate of liberation appearing a long time after administration of a drug is due to some general toxic action rather than to stimulation of

a secretory mechanism notable for the promptitude with which it responds to appropriate excitation.

This suggestion is supported by the fact that whenever an apparent small increase has been seen in the output in adrenal blood specimens collected soon after the injection of strophanthin, the dose has been such as to kill the animal very quickly thereafter. For example, in cat 296, although the dose (0.05 mgm. per kilogram) was slightly smaller than in cat 290, the animal died very soon after the end of collection of the fourth specimen, four to five minutes after the administration of the drug. A moderate increase in the output was found, both in the third and fourth specimens, although it was still well within the normal range. The assay was such that an increase of less than 50 per cent could be certainly demonstrated and the calculated increase was somewhat more than this. There is no doubt then that there was a small increase in the output in this experiment, beginning half a minute to a minute after the injection of the drug and still present two to four minutes after the injection. But compared with the augmentation caused by strychnine or the transient augmentation caused by nicotine, the effect, even if in this experiment it was directly due to the strophanthin, is insignificant.

Condensed protocol. Cat 296; female; weight, 2.0 kgm.

Anesthetized with ether. Obtained specimen of indifferent blood from jugular. Cut vagi. Made cava pocket. Collected adrenal blood.

10.40 a.m. First specimen, small—not weighed.

10.40½ a.m. Second specimen, 3.7 grams in 240 seconds (0.92 gram per minute). Blood pressure at beginning of collection of second adrenal specimen was 74 mm. of mercury (fig. 13, observation 3); at the end of collection of second specimen 64 mm. (observation 4).

10.51 a.m. Injected intravenously 0.1 mgm. strophanthin (fig. 13, observations 5 to 6).

10.51½ a.m. Third adrenal specimen, 2.6 grams in 90 seconds (1.7 grams per minute).

10.53 a.m. Fourth adrenal specimen, 2.85 grams in 150 seconds (1.14 grams per minute). Blood pressure at beginning of collection of third specimen was 100 mm. of mercury (fig. 13, observation 7); at beginning of collection of fourth specimen 82 mm. (observation 8); at end of collection of fourth specimen 56 mm. (observation 9). Shortly after the collection of fourth adrenal specimen the heart became irregular and soon stopped. Combined weight of adrenals 0.215 gram.

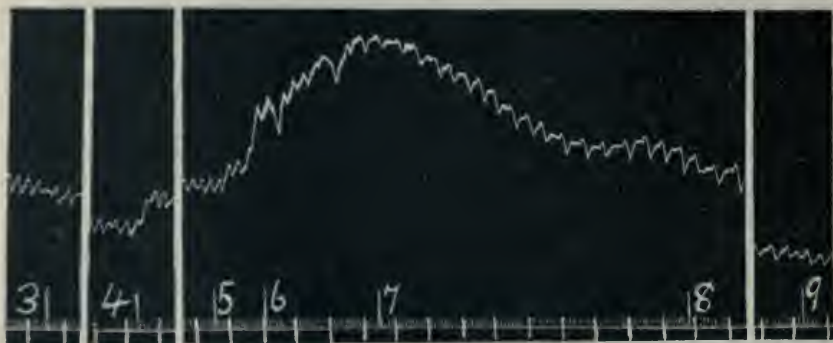


FIG. 13. BLOOD PRESSURE TRACING. CAT 296

3 and 4 The beginning and end of collection of second adrenal specimen; 5 to 6, intravenous injection of strophanthin; 7, beginning of collection of third adrenal specimen; 8, beginning of collection of fourth adrenal specimen; 9, end of collection of fourth adrenal specimen. Line of zero pressure corresponds with time trace and is moved up 18 mm.

Figure 13 shows the blood pressure at the time the adrenal blood specimens were procured. In figures 14 to 16 are reproduced some of the tracings used in the assay. It was shown that the second specimen, collected before injection of strophanthin, was weaker than 1:1,660,000 adrenalin, weaker than 1:2,100,000, stronger than 1:2,500,000 (fig. 14, confirmed by other observations not reproduced). It was taken at 1:2,300,000, corresponding to an output of 0.0004 mgm. per minute for the cat, or 0.0002 mgm. per kilogram per minute.

The third specimen was much weaker than 1:1,660,000, much weaker than the fourth specimen (fig. 15), not very different in

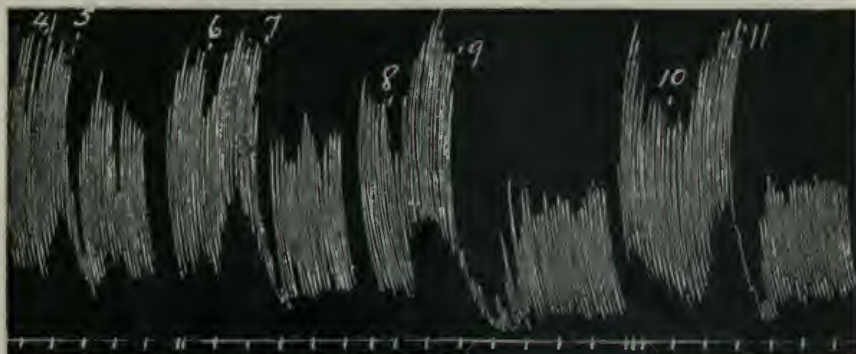


FIG. 14. INTESTINE TRACINGS. BLOODS FROM CAT 296

At 4, 6, 8 and 10, Ringer was replaced by jugular blood; and this at 5 by jugular blood to which was added adrenalin to make a concentration of 1:2,500,000; at 7 by the second adrenal specimen (collected before injection of strophanthin); at 9 by jugular blood to which was added adrenalin to make a concentration of 1:1,660,000; at 11 by jugular blood to which was added adrenalin to make a concentration of 1:2,100,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

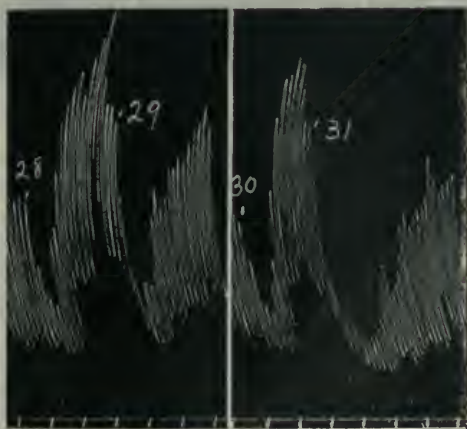


FIG. 15. INTESTINE TRACINGS. BLOODS FROM CAT 296

At 28 and 30 Ringer was replaced by jugular blood to which was added strophanthin (0.001 mgm. to each 1.25 cc. of blood); and this at 29 by the third adrenal specimen (collected immediately after injection of strophanthin); at 31 by the fourth adrenal specimen (collected two minutes after injection of strophanthin). All the bloods were diluted with three volumes Ringer. Reduced to one-half.

concentration from the second specimen, but slightly weaker. It was taken at 1:2,500,000, equivalent to an output of 0.0007 mgm. per minute for the cat, or 0.00035 mgm. per kilogram per minute. The fourth specimen was much stronger than 1:2,500,000, much stronger than the second specimen (fig. 16, observations 19 and 21), little different from 1:1,660,000 (fig. 16, observations 19 and 25, confirmed by other observations not reproduced), but probably slightly stronger. Taking it at 1:1,600,000, we get 0.0007

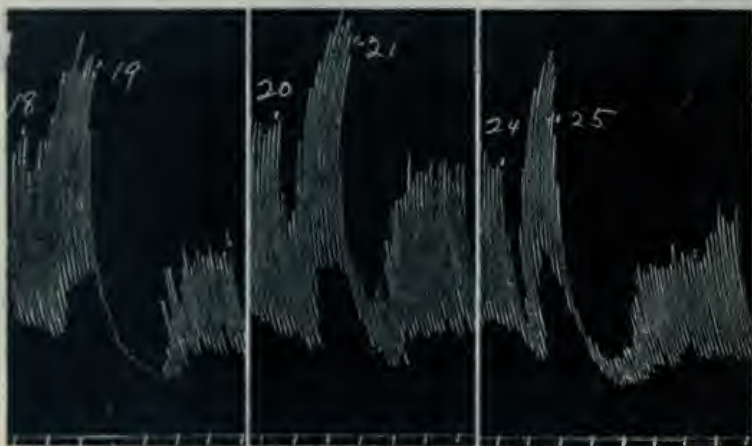


FIG. 16. INTESTINE TRACINGS. BLOODS FROM CAT 296

At 18, 20 and 24 Ringer was replaced by jugular blood and this at 19 by the fourth adrenal specimen (collected two minutes after injection of strophanthin); at 21 by the second adrenal specimen (collected before injection of strophanthin); at 25 by jugular blood to which was added adrenalin to make a concentration of 1:1,660,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

mgm. per minute for the cat, or 0.00035 mgm. per kilogram per minute, the same output as for the third specimen.

The two following protocols (cats 317 and 319) illustrate the observations in which the strophanthin was injected slowly.

Condensed protocol. Cat 317; female; weight, 2.0 kgms.

Anesthetized with ether. Obtained indifferent blood from jugular.
Cut vagi. Made cava pocket. Collected adrenal blood.

- 10.10 a.m. First specimen, 2.3 grams in 30 seconds (4.6 grams per minute).
- 10.10½ a.m. Second specimen, 7.65 grams in 2 minutes (3.82 grams per minute). Blood pressure at beginning of collection of second specimen 130 mm. of mercury, at end of collection 110 mm.
- 10.23 to 10.35½ a.m. Injected intravenously 0.1 mgm. strophanthin.
- 10.36½ a.m. Third adrenal specimen, 2.15 grams in 30 seconds (3.05 grams per minute).
- 10.37 a.m. Fourth adrenal specimen, 6.1 grams in 2 minutes (3.05 grams per minute). Blood pressure at beginning of collection of third specimen 136 mm. of mercury; at beginning of collection of fourth specimen 130 mm.; at end of fourth specimen 100 mm.
- 10.53 a.m. Fifth adrenal specimen, 1 gram in 30 seconds (2 grams per minute).
- 10.53½ a.m. Sixth adrenal specimen, 4.1 grams in 3 minutes (1.4 grams per minute). Blood pressure at beginning and end of collection of fifth specimen 76 mm. of mercury; at end of sixth specimen 48 mm.

Weight of adrenals 0.29 gram.

The second specimen, collected before administration of the drug, was found to have a smaller concentration of epinephrin than the fourth, collected at the end of the strophanthin injection, and the fourth, a smaller concentration than the sixth, procured about twenty minutes after the injection. The detailed assay showed that the second specimen was much weaker than 1:2,750,000 adrenalin, decidedly weaker than 1:7,000,000, distinctly stronger than 1:10,000,000 (confirmed by several sets of observations). It was taken at 1:9,000,000, corresponding to an output of 0.00042 mgm. per minute for the cat, or 0.00021 mgm. per kilogram per minute.

The fourth specimen was stronger than 1:7,000,000, weaker than 1:5,500,000, approximately the same as 1:6,000,000, equivalent to an output of 0.0005 mgm. per minute for the cat, or 0.00025 mgm. per kilogram per minute. The sixth specimen was much stronger than 1:5,500,000, much weaker than 1:1,750,000, somewhat weaker than 1:2,750,000. It was taken at 1:3,000,000

corresponding to an output of 0.00047 mgm. per minute for the cat, or 0.00023 mgm. per kilogram per minute. In this animal accordingly no change in the epinephrin output could be demonstrated after strophanthin.

Condensed protocol. Cat 319; male; weight, 2.3 kgms.

Anesthetized with ether. Obtained indifferent blood from jugular. Cut vagi. Made cava pocket. Collected adrenal blood.

10.16½ a.m. First specimen, 1.05 grams in 30 seconds (2.1 grams per minute).

10.17 a.m. Second specimen, 5.1 grams in 180 seconds (1.7 grams per minute). Blood pressure at end of collection of second specimen was 76 mm. of mercury, at beginning 90 mm.

10.27½ a.m. Beginning of injection of 0.1 mgm. strophanthin.

10.40 a.m. End of injection of strophanthin. Blood pressure at end of injection of strophanthin was 112 mm. of mercury.

10.41 a.m. Third adrenal specimen, 1 gram in 30 seconds (2 grams per minute).

10.41½ a.m. Fourth adrenal specimen, 5.45 grams in 240 seconds (1.36 grams per minute). Blood pressure at beginning of collection of third specimen was 94 mm. of mercury; at beginning of collection of fourth specimen 89 mm.; at end of collection of fourth specimen 67 mm.

11.04½ a.m. Fifth adrenal specimen, 30 seconds collection—not weighed.

11.05 a.m. Sixth adrenal specimen, 2.55 grams in 270 seconds (0.57 gram per minute). Blood pressure at beginning of collection of sixth specimen was 59 mm. of mercury; at end of collection 47 mm.

Obtained another specimen of venous blood. Combined weight of adrenals 0.301 gram.

The second specimen, collected before administration of strophanthin, was shown to have a concentration of epinephrin much less than 1:3,750,000 and somewhat less than 1:5,000,000. It was taken at 1:5,750,000, corresponding to an output of

0.0003 mgm. per minute for the cat, or 0.00013 mgm. per kilogram per minute.

The fourth specimen, procured immediately after the end of the strophanthin injection was found to be much weaker than 1:2,500,000, and somewhat weaker than 1:3,750,000 (confirmed by several pairs of observations). It was finally taken at 1:4,000,000, corresponding to an output of 0.00034 mgm. per minute for the cat, or 0.00014 mgm. per kilogram per minute.

The sixth specimen, collected about half an hour after the end of the strophanthin injection was decidedly weaker than 1:1,250,000, and approximately the same as 1:1,875,000 adrenalin (confirmed by several sets of observations). Taking it at 1:1,875,000, we get 0.0003 mgm. per minute as the epinephrin output for the cat, or 0.00013 mgm. per kilogram per minute. Therefore, no change whatever could be demonstrated in this animal after administration of the drug.

The last experiment with rabbit segment assays which will be referred to, was made on a dog (297) in which the brain stem was divided at the cerebral peduncles. This was done because in the experiments of Richards and Wood the usual procedure was to begin with a brain mutilation which rendered the subsequent administration of an anesthetic unnecessary. As already mentioned, however, our experience is that such interference with the central nervous system may introduce such complications that it should be avoided as a routine measure in experiments on the epinephrin output. The experiment to be described is a good illustration of this.

Condensed protocol. Dog 297; female; weight, 5.09 kgm.

Anesthetized with ether. Through a trephine opening cut across the cerebral peduncles and discontinued anesthetic; respirations were good, but for uniformity of the blood pressure curve started artificial respiration. Obtained specimen of indifferent blood from jugular. Made cava pocket. Collected adrenal blood.

10.39 a.m. First specimen, 2.9 grams in 30 seconds (5.8 grams per minute).

- 10.39 $\frac{1}{2}$ a.m. Second specimen, 8.55 grams in 120 seconds (4.3 grams per minute). Blood pressure at beginning of collection of second adrenal specimen was 55 mm. of mercury.
- 10.47 a.m. to 10.47 $\frac{3}{4}$ a.m. Injected intravenously 0.4 mgm. strophanthin in doses of 0.25 mgm. and 0.15 mgm.
- 10.49 a.m. Third adrenal specimen, 4.25 grams in 60 seconds (4.25 grams per minute).
- 10.50 a.m. Fourth adrenal specimen, 6.55 grams in 120 seconds (3.28 grams per minute). Blood pressure at beginning of collection of third adrenal specimen was 57 mm. of mercury; at beginning of collection of fourth specimen 54 mm.; at end of collection of fourth specimen 45 mm.
- 11.02 a.m. Fifth adrenal specimen, 0.85 gram in 60 seconds (0.85 gram per minute).
- 11.03 a.m. Sixth adrenal specimen, 2.3 grams in 240 seconds (0.6 gram per minute). Blood pressure at beginning of collection of fifth adrenal specimen was 34 mm. of mercury; at beginning of collection of sixth specimen 28 mm.

Obtained another specimen of venous blood. Combined weight of adrenals 0.883 gram.

Some of the tracings used in the epinephrin assay are reproduced in figures 17 to 20. The second adrenal blood specimen, collected before the administration of strophanthin, the blood pressure being 55 mm. of mercury, was found to be much stronger than 1:5,000,000 (observations not reproduced), or than 1:2,500,000 (fig. 17, observations 7 and 9), stronger than 1:1,250,000 (fig. 17, observations 9 and 13), and very much the same as 1:830,000 (fig. 17, observations 9 and 11). These results were corroborated by other intestine tracings (not reproduced). It was confirmed by uterus tracings (fig. 18) that the second adrenal specimen was stronger than 1:1,250,000. The indifferent (jugular) blood in the same dilution gave only an insignificant effect on the uterus segment. Taking the second specimen at 1:830,000, we get an epinephrin output of 0.0052 mgm. per minute for the dog, or 0.001 mgm. per kilogram per

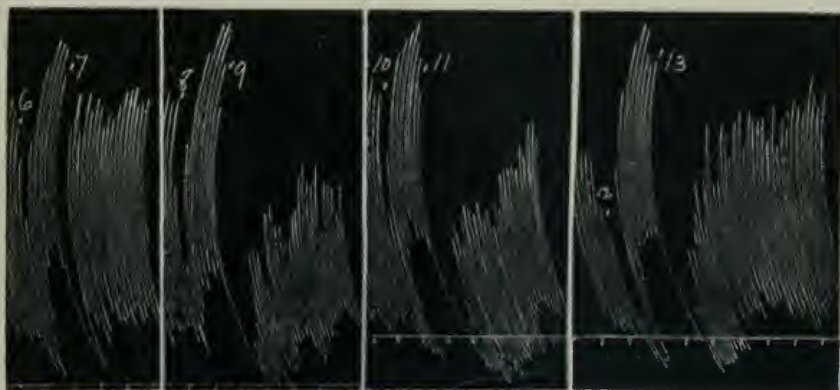


FIG. 17. INTESTINE TRACINGS. BLOODS FROM DOG 297

At 6, 8, 10, and 12 Ringer was replaced by jugular blood and this at 7 by jugular blood to which was added adrenalin to make a concentration of 1:2,500,000; at 9 by the second adrenal specimen (collected before injection of strophanthin); at 11 by jugular blood to which was added adrenalin to make a concentration of 1:830,000; at 13 by jugular blood to which was added adrenalin to make a concentration of 1:1,250,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to two-fifths.

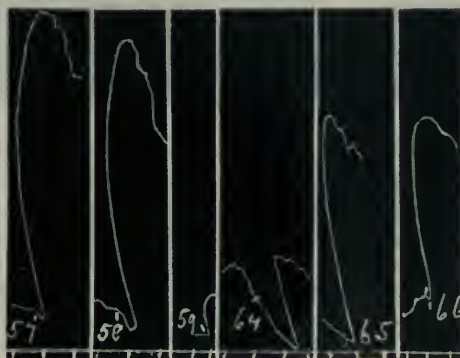


FIG. 18. UTERUS TRACINGS. BLOODS FROM DOG 297

At 57 Ringer was replaced by the second adrenal specimen (collected before injection of strophanthin); at 58 by jugular blood to which was added adrenalin to make a concentration of 1:1,250,000; at 59 and 64 by jugular blood; at 65 by the second adrenal specimen; at 66 by the third adrenal specimen (collected immediately after injection of strophanthin). All the bloods were diluted with ten volumes Ringer (the adrenalin blood after adding the adrenalin). Observations 64, 65 and 66 were taken with a smaller magnification than the other observations. Reduced to one-half.

minute. This is more than four times the average in dogs under ordinary anesthesia (4) and much beyond the maximum range, suggesting that the brain operation either had removed an inhibitory influence on the secretion, exercised by some portion of the brain above the lesion, or that the irritative effects of the lesion had increased the output. We have seen indications in our work on strychnine (5) that this drug may, under certain

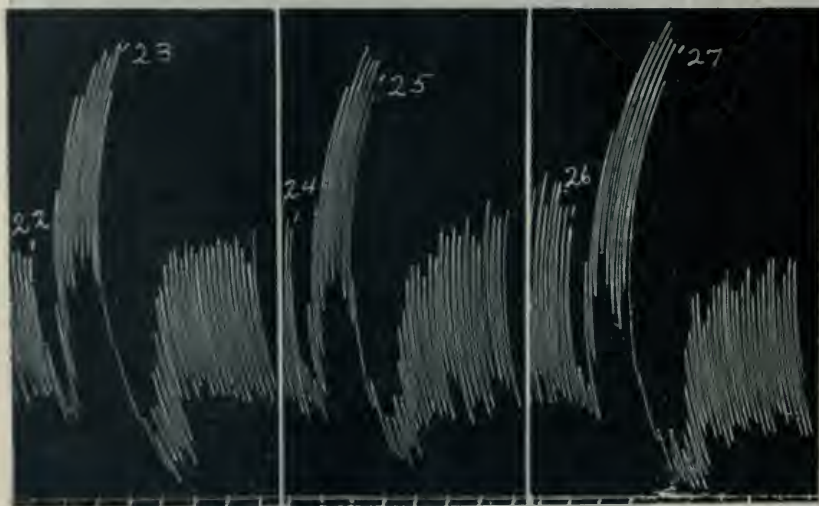


FIG. 19. INTESTINE TRACINGS. BLOODS FROM DOG 297

At 22, 24 and 26 Ringer was replaced by venous blood (collected after injection of strophanthin); and this at 23 by the third adrenal specimen (collected immediately after injection of strophanthin); at 25 by the venous blood to which was added adrenalin to make a concentration of 1:830,000; at 27 by the venous blood to which was added adrenalin to make a concentration of 1:660,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

conditions, cause a transient diminution of the epinephrin discharge preceding the marked and prolonged augmentation which is its predominant action, and it is possible that this effect may be due to a brief excitation by strychnine of an inhibitory mechanism situated at a higher level than the center whose stimulation causes the increased liberation. Elliott (6) has

shown that such brain mutilations are associated with a decided diminution in the epinephrin store of the adrenals, and although, as we have several times pointed out, a diminution in the epinephrin store is not of itself evidence that the epinephrin discharge has been augmented, such a diminution in the store may very well, in certain cases, be associated with an increased

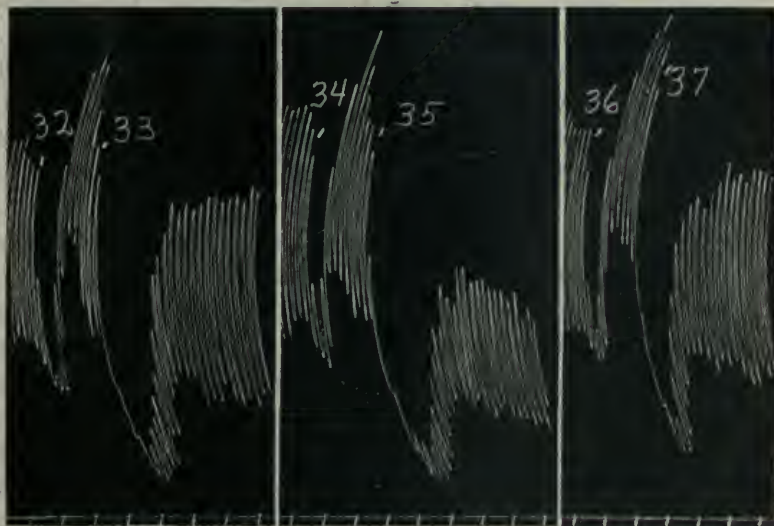


FIG. 20. INTESTINE TRACINGS. BLOODS FROM DOG 297

At 32, 34, and 36 Ringer was replaced by venous blood (collected after injection of strophanthin); and this at 33 by the fourth adrenal specimen (collected two minutes after injection of strophanthin); at 35 by the venous blood to which was added adrenalin to make a concentration of 1: 660,000; at 37 by the venous blood to which was added adrenalin to make a concentration of 1: 830,000. All the bloods were diluted with three volumes Ringer (the adrenalin bloods after adding the adrenalin). Reduced to one-half.

output, due either to removal of an inhibitory influence, or to the production of a continuous stimulation.

The third adrenal specimen, the collection of which was begun a little more than a minute after completion of the strophanthin injection, had practically the same concentration of epinephrin as the second. On the intestine segment it was shown to be much stronger than 1: 1,250,000, stronger than 1: 1,000,000

(confirmed by several observations), probably slightly stronger than 1:830,000 (fig. 19, observations 23 and 25), but decidedly weaker than 1:670,000 (fig. 19, observations 23 and 27). Taking the third specimen at 1:800,000, we get 0.0053 mgm. per minute for the dog, or 0.001 mgm. per kilogram per minute, the same as for the specimen before injection of strophanthin.

The fourth adrenal specimen, beginning about two minutes after the strophanthin injection, had very much the same concentration as the third and the second. It was probably slightly stronger than 1:830,000, but markedly weaker than 1:670,000 (fig. 20). Taking it at 1:800,000, we get an output of 0.00041 mgm. per minute for the dog, or 0.0008 mgm. per kilogram per minute.

The sixth adrenal specimen, obtained fifteen minutes after injection of strophanthin, was found to be decidedly stronger than 1:830,000, but weaker than 1:670,000 (confirmed by several observations). It was taken at 1:750,000, corresponding to an output of 0.0008 mgm. per minute for the dog, or 0.00016 mgm. per kilogram per minute. The proportion of serum in the blood was 51 per cent, as determined by the electrical method, and 48 per cent by the haematocrite (after fifty minutes rotation). The epinephrin concentration in the serum of the sixth specimen must, therefore, have been somewhat greater than 1:400,000. It is obvious that with the slow blood flow when the sixth specimen was collected, the calculated output could not possibly equal that for the preceding specimens, unless strophanthin possessed the power, like nicotine, of pushing up the possible maximum concentration far beyond the normal limit.

In reviewing the experiments in which the adrenal blood was assayed on rabbit segments, it is obvious that if strophanthin in the doses employed by us influences the epinephrin output at all, the effect is of a different order of magnitude from that produced by strychnine or from the transient excitation caused by nicotine. And while a marked effect can be elicited by strychnine and nicotine with great constancy, no increase was found in any adrenal blood specimen taken after strophanthin in at least two-thirds of the experiments. In one or two ani-

mals no increase was found in the specimens taken immediately or a few minutes after the administration of the drug, and while it was maintaining a rise of blood pressure, while a remote specimen, collected many minutes thereafter, gave a slightly increased output. In one or two of the animals a slight increase was found in specimens obtained soon after the injection of strophanthin. In no case, either in near or remote specimens, did such an increase as was detected suffice to raise the output beyond the normal range seen in animals under the influence of an ordinary anesthetic alone. The observed increases never amounted to more than 50 to 100 per cent of the initial output before strophanthin, and it is only with rather favorable conditions that changes of much less than 50 per cent in the normal output can be certainly detected with the method employed.

We should hesitate to attribute the occasional small changes observed in the rate of epinephrin output to a stimulating action of strophanthin upon the adrenal secretory mechanism. Whether with a more delicate method, if such should be developed, it would be possible to demonstrate a definite effect of strophanthin on this mechanism, we can, of course, offer no opinion. It appears to us, however, quite likely that a good many drugs, especially such as are known to exert an action upon sympathetic fibers, or on organs supplied by them, may influence the epinephrin output, although not necessarily in a degree detectable or measurable by the best of our present methods.

EXPERIMENTS WITH AUTO-ASSAY BY BLOOD PRESSURE REACTIONS

The experiments with direct collection of adrenal blood and assay of the epinephrin on rabbit segments were controlled by three experiments in which auto-assays by blood pressure reactions were attempted. In one of these, a female cat (314) weighing 2.12 kgm., under urethane, the output of epinephrin was estimated at about 0.0002 mgm. per kilogram per minute by several pocket observations. Strophanthin (0.05 mgm.) was then injected into the jugular vein, the cava pocket closed a few seconds thereafter and adrenal blood collected in the pocket for

two minutes. The effect on the blood pressure of releasing the pocket was about the same as that due to injection (while the pocket was closed off) of 0.5 cc. of a 1:475,000 adrenalin solution, corresponding to an output of about 0.00025 mgm. per kilogram per minute. Another dose of 0.05 mgm. strophanthin was given eight minutes after the first. The strophanthin was put in just after the cava pocket had been closed off. The pocket was kept closed for two minutes and its release produced an effect on the blood pressure somewhat less than that caused by 0.5 cc. of 1:475,000 adrenalin, but decidedly greater than that caused by 0.5 cc. of 1:950,000 adrenalin. The output of epinephrin was, therefore, somewhat less than 0.00025 mgm. per kilogram per minute. Other observations in this experiment showed no increase whatever as compared with the original output before strophanthin. In some observations the output appeared to be decreased rather than increased. A third dose of strophanthin (0.05 mgm.) given thirteen minutes after the second, quickly killed the animal. The adrenals weighed 0.587 gram. The vagi were cut at the beginning of the experiment.

In the second experiment of this group (cat 318) the strophanthin (0.15 mgm. in a 3.3 kgm. male cat) was slowly injected (during ten minutes). The animal was anesthetized with urethane. Release of the cava pocket after being closed for two minutes, before strophanthin, caused a blood pressure reaction much greater than that elicited by injection of 0.25 cc. of 1:130,000 adrenalin, and about the same as that caused by 0.5 cc. of 1:130,000 adrenalin, corresponding to an output of epinephrin of 0.0019 mgm. per minute for the cat, or 0.00057 mgm. per kilogram per minute. A two minute pocket, two to three minutes after the end of the strophanthin injection, gave an effect somewhat greater than that produced by 0.5 cc. of 1:130,000 adrenalin, but much less than that produced by 0.5 cc. of 1:65,000. Another observation, ten minutes after completion of the strophanthin injection, showed that the effect of releasing a pocket after two minutes closure was less than that of injecting 0.5 cc. of 1:100,000 adrenalin and no greater than, if as great as that caused by 0.5 cc. of 1:130,000 adrenalin (confirmed by another observation). A one minute pocket elicited

about the same effect as a one minute pocket before strophanthin. In other words, up to this time no increase in the output had occurred. Release of a two minute pocket, twenty-five minutes after the end of the strophanthin injection caused a decidedly smaller effect than injection of 0.5 cc. of 1:100,000 adrenalin. The effect produced when a pocket which had been closed for three minutes was released was somewhat less than that produced by 0.5 cc. of 1:65,000 epinephrin, but greater than that produced by 0.5 cc. of 1:100,000 adrenalin. Taking the epinephrin collected in the pocket in three minutes as equivalent to 0.5 cc. of 1:80,000 adrenalin, we get 0.002 mgm. per minute as the output for the cat, or 0.0006 mgm. per kilogram per minute, the same as before strophanthin. The adrenals weighed 0.367 gram.

In the third experiment, with blood pressure auto-assay (cat 313), the animal, a female, weighing 3 kgm. was anesthetized with ether. The vagi were cut. Six pocket observations were made before strophanthin was injected, and the output of epinephrin was estimated at about 0.0003 mgm. per kilogram per minute. Strophanthin (0.05 mgm.) was then injected into the jugular vein immediately after the closing off of the pocket. The blood pressure reaction, on releasing it after two minutes closure, seemed qualitatively somewhat increased. This was confirmed by another pocket observation. But it could not be quantitatively shown by comparison with the effects now produced by given doses of adrenalin that there was an increase in the rate of output. A second dose of strophanthin (0.05 mgm.) was then given, thirteen minutes after the first, and the pocket closed in fifteen seconds. No good assay could be made on account of irregularity in the blood pressure curve. But the output was not much, if at all above 0.0003 mgm. per kilogram per minute. The intestinal arteries are usually not tied in such experiments because the preservation of the splanchnic area is favorable to a large epinephrin effect.

The experiments with blood pressure assay leave us accordingly in the same position as those with assay on the rabbit segments, that is, without real evidence of any definite and constant effect of the drug upon the epinephrin output.

SUMMARY

We have been unable to demonstrate any decided and constant effect of strophanthin upon the epinephrin output. Statements in the literature that the drug causes a marked augmentation of the output are based upon the use of inadequate methods.

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DEMONSTRATION THAT THE SPONTANEOUSLY LIBERATED EPINEPHRIN CAN EXERT AN ACTION UPON THE HEART

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In the course of our experiments upon the epinephrin output, evidence has accumulated that the steady spontaneous discharge is of sufficient magnitude to cause definite physiological effects in the organism. For example, in the latter part of the experiment on cat 313 in the paper on strophanthin a series of observations were made which constitute a demonstration that the epinephrin discharged from the adrenals at the ordinary rate can exert a clearly detectable action upon the heart. We have other experiments in which strophanthin was not given which prove the same thing and one of them will also be referred to, as it seems to us more important to establish that the amount of epinephrin spontaneously given off can and does produce definite physiological reactions, than to deduce from the effects of the monstrous doses of adrenalin which have been employed in investigating its so-called "physiological action" what epinephrin must do when liberated naturally into the blood stream.

In the experiment on cat 313 about eight minutes after the second dose of strophanthin an irregularity developed in the blood pressure tracing, which was not connected with any change in the respiration. The animal was breathing naturally and artificial respiration had not been employed. At this time an injection of 0.5 cc. of 1:150,000 adrenalin was made into the jugular vein (fig. 1, observation 34). The irregularity, which was unquestionably due to a cardiac arrhythmia, became more pronounced. At 35, one minute after the adrenalin injection,

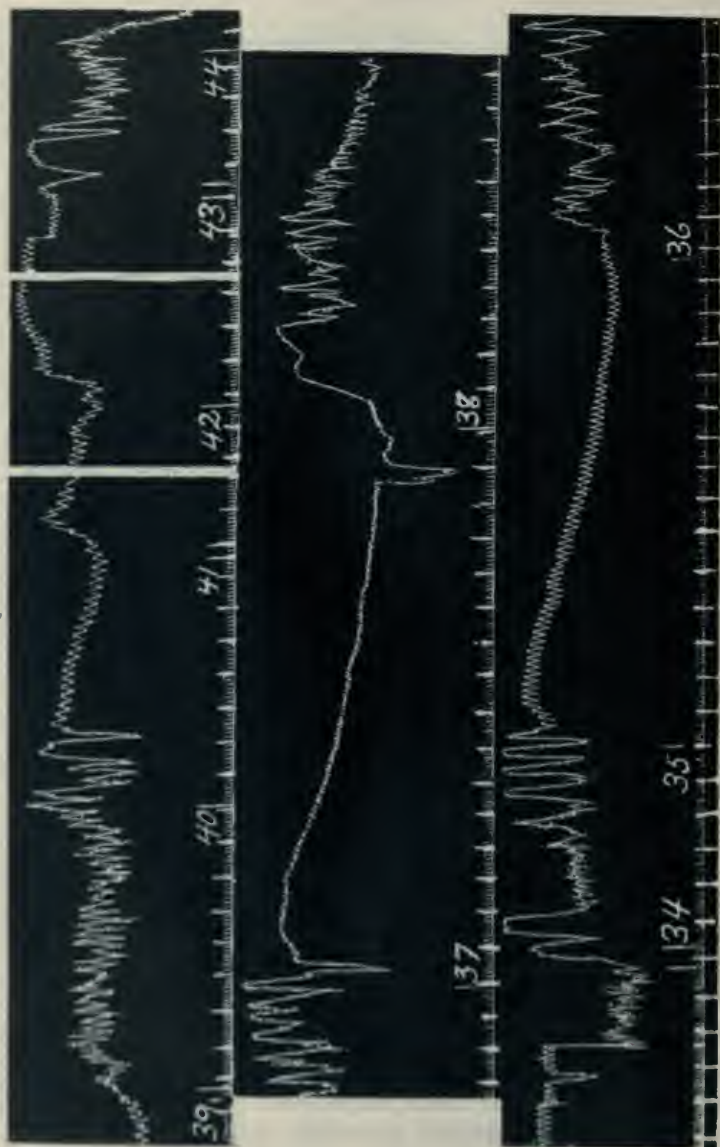


FIG. 1. BLOOD PRESSURE TRACING FROM CAT 313, AFTER ADMINISTRATION OF STROPHANTHIN

Showing the effect of exclusion of the naturally liberated epinephrin upon the cardiac irregularity, and demonstrating that the epinephrin discharged from the adrenals exerts an action upon the heart. For details, see text. The zero line, coincident with the time trace, has been moved up 30 mm. for observations 34 to 36; 23 mm. for observations 37 and 38; and 28 mm. for observations 39 to 44. Time, seconds and 8 seconds.

the cava pocket was closed off and the irregularity completely disappeared after a latent interval, allowing for lost time in making the signal mark, of six or seven seconds. During the two minutes for which the pocket was closed, no trace of the irregularity could be discerned. The exclusion of the epinephrin which had previously been entering the blood at the rate of about 0.001 mgm. per minute, must accordingly have been responsible for the marked change in the heart's action, and, therefore, this epinephrin discharge must have previously been exerting an effect upon the heart which contributed to the arrhythmia. The action of the previously injected adrenalin, if this had anything to do with the intensification of the arrhythmia, as it doubtless had from other observations made later, would almost certainly have passed off before the pocket was closed, as shown by the duration of the effect of adrenalin in other observations on this cat. But it is a matter of indifference as regards the cogency of the demonstration whether this was the case or not, since the experiment was repeated again and again without any further injection of adrenalin and with the same result. At 36 (fig. 1) the pocket was opened, the accumulated epinephrin entered the circulation and after a latent period of seven or eight seconds, the irregularity reappeared. The entrance of the accumulated epinephrin was, therefore, responsible for the reestablishment of the cardiac irregularity. This would not prove anything as to the action of the naturally liberated epinephrin *discharged at its normal rate* on the heart, since it had been accumulated for two minutes and was then allowed to enter the blood stream rather abruptly. But now at 37, when this accumulated epinephrin must have long since disappeared, the pocket was again closed, and again after about the same latent period the heart began to beat regularly, showing that the arrhythmia was conditioned by the epinephrin discharged at the ordinary rate from the adrenals. The pocket was kept closed for two minutes. Towards the end of this period in spite of the exclusion of the epinephrin, a brief recurrence of the irregularity took place, but this passed off and did not recur till after the opening of the pocket at 38, after a latent period

of about twenty seconds. At this stage the tendency to arrhythmia was less pronounced than earlier and in about a minute after the opening of the pocket the heart was beating with fair, although not perfect regularity. At this point, one and a half minutes after the opening of the pocket, 0.5 cc. of a 1:300,000 solution of adrenalin was injected into the jugular (observation 39), markedly intensifying the irregularity after a brief latent period. A minute later the pocket was closed at 40 and after an interval of somewhat more than fifteen seconds the curve again became regular. At 41, 0.5 cc. of 1:300,000 adrenalin was again injected, while the pocket was still closed, but now the heart was in such a condition that no noticeable irregularity was produced. The pocket was opened at 42, after having been closed for two minutes. The naturally secreted epinephrin accumulated during this time, although it caused a fair rise of blood pressure, produced, like the artificially introduced adrenalin, no irregularity at this stage. Two minutes later (at 43) a third dose of strophanthin (0.05 mgm.) was injected, developing at once a marked cardiac irregularity. At 44 the cava pocket was closed off, but the blood pressure fell rapidly and the animal died.

The conclusion which we wish to draw from this experiment and which we think follows inevitably, is that the naturally liberated epinephrin entering the circulation at its normal rate was producing a demonstrable effect upon the heart. The precise nature of the action and the precise point of attack of the epinephrin need not be discussed at present. But it seems fairly obvious that the strophanthin arrhythmia was heightened, or the threshold of the strophanthin, action lowered by the naturally discharged epinephrin. At a stage when the strophanthin action had worn off and adrenalin, in such doses as are employed for assaying the amount of the natural output, no longer developed the irregularity, the naturally secreted epinephrin was equally without effect.

It does not necessarily follow from these observations that the susceptibility of the heart is increased by strophanthin for all the reactions which epinephrin in the amounts in which it is normally liberated can exert upon the heart. But if this were the

case for the accelerating affect, for instance, it is easy to see that the consideration which led Richards and Wood to suspect that strophanthin might increase the epinephrin output would bear no such significance. They state that they were led to this idea by observing that the accelerating effect of strophanthin upon the heart was much more common with the heart in situ than with the isolated organ. There are, of course, many possible causes for such a difference between an isolated organ and the same organ in situ. But if the epinephrin discharged from the adrenals has anything to do with it, it might be explained equally well on the hypothesis that the naturally secreted epinephrin produced the effect, not because it was liberated at an augmented rate but because the test object had been rendered more sensitive to it. That the adrenal vein blood when collected in a cava pocket and then released, causes a marked acceleration of the heart in animals to which no drug except an ordinary anesthetic has been administered, is easy to demonstrate. To show an effect of the steady normal discharge of the epinephrin on the heart rate is another matter. As we have already pointed out in the case of the blood pressure a negative result of exclusion of the adrenal blood when the regulative nervous mechanism is still intact has little value.

It is not only in animals under the influence of strophanthin that the normally secreted epinephrin has been shown to have an influence on the heart. In figure 2 are reproduced portions of a blood pressure tracing from a cat (265) anesthetized with urethane, which exhibits phenomena fully as striking and incapable of any other explanation than that the epinephrin discharged steadily into the blood stream by the adrenals at a rate well within the normal range was exerting a definite effect upon the heart. The animal, a male, weighing 3.78 kgm., received 6 grams urethane by stomach tube and was deeply anesthetized in half an hour. The vagi were cut at the beginning of the experiment. It was noted at the beginning on palpating the pulse that the heart was irregular and that every few beats a pulsation seemed to be dropped. It was estimated by the method of auto-assay by blood pressure reactions, that the epinephrin

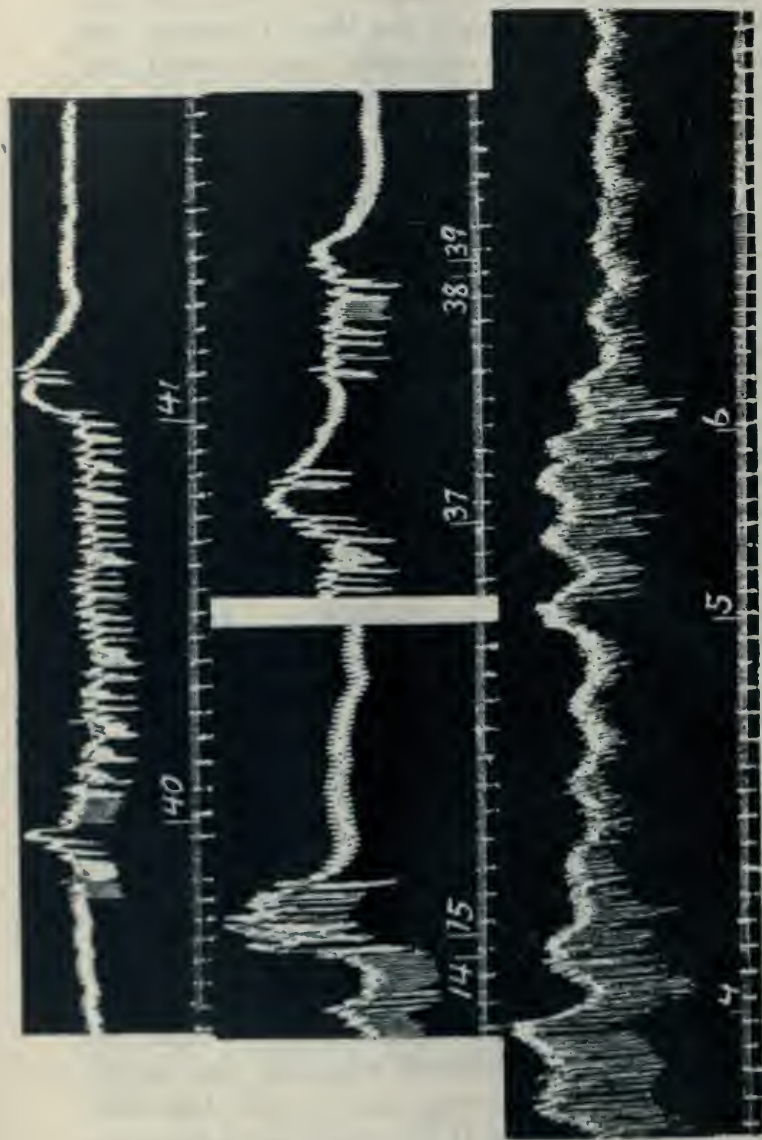


FIG. 2. BLOOD PRESSURE TRACING FROM CAT 265, ANESTHETIZED WITH URETHANE

Showing the effect of exclusion of the naturally liberated epinephrin on a cardiac arrhythmia present at the beginning of the experiment, and demonstrating that the epinephrin discharged from the adrenals exerts an action upon the heart. For details, see text. The zero line, coincident with the time trace, has been moved up 42 mm. for observations 4 to 6; 40 mm. for observations 14 and 15; and 20 mm. for observations 37 to 41. In this figure, as in figure 1, the finer details of the irregularity in the curve have been somewhat obscured in the reproduction.

output was not more than 0.0002 mgm., or less than 0.00015 mgm. per kilogram per minute, i.e., rather under than above the normal average. A more exact assay could not be made on account of the irregularity of the heart. The adrenals weighed 0.755 gram. It was observed that on closing off the cava pocket, the appearance of the blood pressure curve altered in such a way as to indicate a marked diminution in the cardiac irregularity, as shown for instance in figure 2 at 4 where the pocket was closed. The change could have nothing to do with any effect which the abstraction of such a small quantity of blood as was collected in the pocket (about 1 to at most 2 cc.) could have had on the filling of the heart. The latent period of the effect (eight or ten seconds) was such as would be expected if it was due to the disappearance of an action on the heart which was being exerted by the small steady output of epinephrin into the blood stream before the closing of the pocket. A similar pocket observation earlier in the experiment gave the same result. At 5 the pocket was released, the pent up adrenal blood passed into the circulation and after the usual latent period of eight or ten seconds, the irregularity of the heart beat returned. At 6 the pocket was again closed and the curve altered precisely as before. The same was the case with another pocket observation a few minutes later. The opening of the pocket augmented the irregularity and so did the injection of adrenalin at this stage, in such amounts as were used to assay the epinephrin output. A very large amount of epinephrin, on the other hand (0.5 cc. of a 1: 35,000 solution) caused the irregularity to disappear, perhaps after a brief increase (fig. 2, injection from 14 to 15). Precisely the same reversal of the original effect was seen at a later stage in the experiment when the cava pocket was closed off, the irregularity now developing or becoming greater during the period of closure of the pocket and disappearing, after the usual latent period, when the pocket was opened and the accumulated epinephrin reached the heart. At 37, for example, a pocket which had been closed three minutes, was opened. When the pocket was closed the heart, after the injection of 0.5 cc. of 1: 70,000 adrenalin, a strong dose, was beating regularly. Irregu-

larity developed during the time the pocket was closed and is obvious on the blood pressure curve before 37. After the opening of the pocket the irregularity disappeared for a time, of course after a certain period of delay. The amount of epinephrin collected in the pocket would not be half as much as the dose of adrenalin injected before the closure of the pocket and it did not remove the irregularity for as long a time. At 38 to 39, 1 mgm. of strychnine sulphate was injected intravenously. The irregularity of the heart rapidly disappeared, the period of delay being less than before. The strychnine began to enter the circulation a little before the first signal mark. The second mark indicates the end of the washing in of the dose of strychnine with the usual small quantity of salt solution. There was at this time no noticeable effect on the reflex excitability, but it must be remembered that the cat was deeply anesthetized with urethane. We have shown that strychnine can markedly augment the epinephrin output, and the most natural explanation of the effect upon the heart is that it was due to epinephrin. If the output was augmented, the latent period of the effect would be diminished. A little before 40 the irregularity returned and was decidedly increased by excluding the epinephrin when the pocket was closed at 40. After three minutes, the pocket was opened at 41 and with the arrival of the epinephrin at the heart the irregularity disappeared completely for two to three minutes. When it returned it was much less prominent than before, but was still increased somewhat by closing off the cava pocket and abolished by opening it. This was the case even when the irregularity had been reduced to the occurrence of a group of two or three larger strokes on the blood pressure curve at long intervals. Fourteen minutes after the first dose of strychnine when practically all irregularity had disappeared, another dose of 1 mgm. was given. The reflex excitability was now distinctly increased. Exclusion of the epinephrin caused no return of irregularity until the pocket had been closed for more than two minutes, when three or four small groups of irregular beats were seen at intervals of twelve or fifteen seconds. With the release of the pocket, these groups completely disappeared.

As in the experiment with the strophanthin irregularity we prefer to draw only the conclusion, which seems incontrovertible, that the spontaneously discharged epinephrin was in this animal exerting a demonstrable influence upon the heart. There is nothing really puzzling in the fact that at one stage the exclusion of the epinephrin should diminish or abolish the irregularity, and that the release of the adrenal blood should increase or develop it, while at a later stage in the experiment precisely the opposite effect should be produced. For in a given state of the heart, the amount of epinephrin reaching it from the adrenals may be only sufficient to encourage the development of extra contractions instead of causing an equable acceleration of regular beats. In other words, while inciting the heart to hasten, it may cause it to stumble, whereas with a larger dose of epinephrin or an altered susceptibility of the heart to the same dose the improvement of the working power of the heart by the epinephrin may enable the stumbling heart to rid itself of the arrhythmia while accelerating its beat. The difference would then be something like the difference between the effect of flogging an exhausted and a fresh horse. For our purpose the fact that the response of the heart (as regards the arrhythmia studied) to the exclusion of the naturally liberated epinephrin, was different in the two stages of the experiment, only renders the demonstration of the action of the epinephrin more conclusive. For with the reversal of the response of the heart to artificially injected adrenalin came the reversal of its response to the natural epinephrin discharge. When the adrenal blood is excluded from the circulation other substances than epinephrin, if other substances are liberated from the adrenals into the blood stream, will of course, be excluded also. Such bodies might also affect the heart. But from the exact reproduction by artificially introduced adrenalin of the effects of the adrenal blood upon these cardiac irregularities it is clear that in the phenomena studied it is the epinephrin which is the effective factor.

We have already pointed out that investigations which elucidate the factors concerned in sustaining and modifying the epinephrin output may easily come to have a bearing upon the

output of other adrenal products, e.g., the substance whatever it may be in virtue of which the cortex exerts that action upon the organism which is indispensable for life. On the other hand, it is fairly clear that if the nervous system exerts an influence upon the output of such substances this influence is less complete than in the case of epinephrin.

SUMMARY

It was demonstrated that the epinephrin passing into the blood stream from the adrenals at the ordinary rate can exert a definite action upon the heart. This was clearly shown by the marked effect produced upon the cardiac irregularity evoked by strophanthin, when the adrenal blood was temporarily excluded from the circulation or allowed to enter it. Similar observations were made in a case of cardiac irregularity occurring in the absence of strophanthin. It was proved by the artificial administration of adrenalin that the constituent in the adrenal blood responsible for the observed effects was epinephrin.

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THE EFFECT OF MORPHINE UPON THE ALKALI RESERVE OF THE BLOOD OF DOGS GASSED WITH FATAL CONCENTRATIONS OF CHLORINE¹

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INTRODUCTION

The depressant action of morphine upon the various bodily functions leads, as is well known, to a diminished metabolism, as indicated, for example, by lessened carbonic acid excretion by the lungs. It has, moreover, been considered probable that the alkali reserve of the blood actually becomes increased under the influence of small amounts of morphine. This is indicated by such results of morphine injection as were obtained by Barbour, Maurer and von Glahn (1) who observed in fasting human subjects an increase in the alveolar carbon dioxide; furthermore Underhill, Goldschmidt and Blatherwick (2) have similarly succeeded in producing in normal dogs a change to alkaline in the reaction of the urine.

It was here undertaken, to establish by direct evidence the effect of morphine upon the alkali reserve of the blood, first in normal dogs; secondly in gassed dogs.

METHODS

In all the work herein reported the alkali reserve was measured by the capacity of the blood plasma to absorb carbon dioxide. Experiments 15-22 were performed by the method of Henderson and Morriss (3), while in the remainder of the work

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the Van Slyke (4) method was employed. The air mixtures used for saturating the blood samples contained by analysis approximately 5.5 per cent carbon dioxide.

Blood samples of from 10 to 15 cc. were withdrawn every two to three hours, the total bleeding for one day being about 100 cc.

The dogs were gassed for one-half hour at concentrations of 800 to 900 parts, by volume, of chlorine per million of air according to the standard method of the United States Gas Investigation.

Alkali reserve in normal dogs. Two normal dogs bled every two to three hours exhibited during a period of fifteen hours a carbon dioxide capacity varying within the limits of error allowed by Van Slyke (protocols I and II).

NUMBER	DOG			DATE	TIME	VOLUME PER CENT CO ₂ AT 0°
	Kind	Sex	Weight			
			kgm.		hours	
I	Hound	♂	17.4	11/19/17	0	48.5
					2	50.4
					5	48.5
					7	49.4
					10	48.3
					13	48.0
II	Pointer	♀	16.8	11/19/17	0	56.0
					2	52.3
					5	52.3
					7	52.3
					10	53.9
					13	53.9

Effects of morphine upon the alkali reserve in normal dogs. The following protocols represent two pairs of simultaneously conducted experiments. In the first case (protocols III and IV) two bull dogs were given respectively 10 and 20 mgm. per kilo subcutaneous injections of morphine sulphate. In the second pair of experiments two hounds (protocols V and VI) were given similar doses.

NUMBER	DOG			DATE	TIME	VOLUME PER CENT CO ₂ AT 0°	MORPHINE
	Kind	Sex	Weight				
			kgm.		hours		mgm. per kgm.
III	Bull	♂	11.4	11/16/17	0	46.3	20
					0		
					2	48.0	
					5	50.4	20
					8	54.0	
					8		
					11	51.0	
IV	Bull	♂	10.4	11/16/17	13	51.0	10
					0	45.3	
					0		
					2	45.2	10
					5	50.4	
					8	58.9	
					8		
V	Hound	♂	25.5	11/21/17	11	58.6	20
					13	55.7	
					0	53.2	
					0		20
					2	56.0	
					5	59.8	
					7	61.7	
VI	Hound	♂	25.5	11/21/17	8		10
					11	61.5	
					13	61.4	
					24	61.7	10
					0	53.2	
					0		
					2	53.2	
					5	58.9	
					7	61.7	
					8		
					11	61.5	10
					13	58.6	
					24	59.8	

These results show that the doses of morphine given not only cause a marked increase in the alkali reserve of the blood, but are adequate to maintain the reserve at a high level for many

hours. The larger doses in each case, although causing an earlier onset than the smaller, failed to produce a greater elevation than the latter in the curve of the alkali reserve. A repetition of the injections after eight hours also caused no further increase in the height of the curves.

Effects of morphine upon the alkali reserve of the blood in gassed dogs. Other workers in the Chemical Warfare Service noticed that chlorine gas in concentrations of 800 to 900 parts, by volume, of chlorine per million of air produces within a few hours a marked decrease in the alkali reserve of the blood in dogs. This is likely, however, to be preceded by a considerable increase.

The object of these experiments with morphine was to postpone or prevent the occurrence of this acidosis.

Four pairs of simultaneously conducted experiments will serve to illustrate the effects of morphine upon the alkali reserve in gassed dogs. The pairs were selected with reference to likeness in species, sex and weight. One dog of each pair was gassed as a control, being given no morphine (protocols VII, VIII, IX, X, XI, XII, XIII, and XIV).

Two of the four untreated gassed dogs (VII and XI) exhibited an early increase in the CO_2 capacity of the blood, but within eight hours after gassing all of the gassed controls (VII, IX, XI, and XIV) exhibited the ultimate acidosis.

Of the gassed dogs which received morphine, however, two maintained a high alkali reserve for fourteen hours or longer (VIII and XII). The injection (10 mgm. per kilo) was repeated every twelve hours but both of these animals died within thirty hours. In the other two treated dogs the morphine failed to prevent a prompt fall in the alkali reserve, and early death.

It will be seen from the above experiments that morphine may temporarily influence the alkali reserve of the blood of gassed dogs, but cannot be relied upon to exhibit this effect constantly.

Eight further experiments have sufficed to emphasize the futility of the procedure (protocols XV to XXII). One of the dogs (XVI) was given a double injection (20 mgm. per kilo) but acidosis set in immediately and death occurred within six hours.

NUMBER	DOG			DATE	TIME	VOLUME PER CENT CO ₂ AT 0°	MORPHINE	CHLORINE	FATE
	Kind	Sex	Weight						
			kgm.		hours		mgm. per kgm.	p.p. m.*	
VII	Collie	♀	16.8	11/ 8/17	0	54.0		907	Died
					½-1				
					2	54.1			
					5	57.0			
					8	44.9			
					11	42.4			
VIII	Collie	♀	16.8	11/ 8/17	14-24			886	Died
					0	50.4			
					½-1				
					1½		10		
					2	51.3			
					5	57.8			
					8	60.6			
					11	57.6			
					12		10		
					14	57.6			
IX	Collie	♂	22.3	11/12/17	24		10	866	Recovered
					30				
					0	59.7			
					½-1				
					2	60.5			
					5	56.7			
X	Collie	♂	23.6	11/12/17	8	55.7		825	Died
					11	56.8			
					14	50.7			
					0	47.5			
					½-1		10		
					1.5				
XI	Collie-shepherd	♂	26.8	11/14/17	2	45.3		787	Died
					5	39.5			
					6				
					0	60.7			
					½-1				
					2	70.1			
					5	61.4			Died
					8	60.5			
					11	52.8			
					14	52.8			
					26-28				

* p.p.m. = parts per million.

NUMBER	DOG			DATE	TIME	VOLUME PER CENT CO ₂ AT 0°	MORPHINE	CHLORINE	FATE
	Kind	Sex	Weight						
			kgm.		hours		mgm. per kgm.	p.p.m.	
XII	Shepherd	♂	31.4	11/14/17	0	52.2			
					½-1			851	
					1½		10		
					2	53.9			
					5	62.4			
					8	63.3			
					11	54.8			
					12		10		
					14	51.0			
					25		10		
XIII	Hound	♂	20.9	11/23/17	25-27				Died
					0	53.2			
					½-1			806	
					1½		10		
					2	54.1			
					6	53.2			
					11	30.9			
XIV	Black and tan hound	♂	15.0	11/23/17	11+				Died
					0	55.1			
					1-1½			834	
					2	51.3			
					6	48.5			
					11	36.6			
					11-15				Died

While the other seven maintained a normal or high alkali reserve throughout the first days observations, five of these dogs died on the day following the gassing. A single dog survived the gassing four days, apparently maintaining a high alkali reserve throughout.

With the idea that the antipyretic action of the morphine might detract from its possible value in controlling acidosis or in prolonging life, a number of gassed dogs were subjected to an external temperature of approximately 33°C. to prevent loss of bodily heat. In the constant temperature chamber employed for this purpose, the inflowing air was dried by calcium chloride.

NUMBER	DOG			DATE	TIME	VOLUME PER CENT CO ₂ AT 0°	MORPHINE	CHLORINE	FATE
	Kind	Sex	Weight						
			kgm.		hours		mgm. per kgm.	p.p. m.	
XV	Fox terrier	♀	10.4	10/22/17	0	58.0		911	Died
					0-½				
					1		10		
					2	60.0			
					5	58.4			
					7	72.2			
					12		10		
					26	32.4			
					28		10		
					29	27.5			
					31	27.5			
					32		10		
					32-33				
XVI	Fox terrier	♂	9.1	10/25/17	0	44.0		870	Died
					0-½				
					1½		20		
					2	21.4			
					5	24.8			
					6				
XVII	Irish terrier	♂	9.1	10/26/17	0	42.9		846	Died
					½-1				
					1.5		10		
					2	38.4			
					4	52.8			
					6	52.8			
					7		10		
					23	55.1			
					24		10		
					25	55.7			
					36		10		
					48		10		
					60		10		
					72		10		
					84		10		
					96		10		
					102	61.1			
					108				
XVIII	Tan and white cur	♂	7.7	10/30/17	0	54.0		869	
					½-1				
					2		10		

NUMBER	DOG			DATE	TIME	VOLUME PER CENT CO ₂ AT 0°	MORPHINE	CHLORINE	FATE
	Kind	Sex	Weight						
			kgm.		hours		mgm. per kgm.	p.p. m.	
XVIII	Tan and white cur	♂	7.7	10/30/17	4	59.6			
					6	62.2			
					11		10		
					12-24				Died
XIX	Coach	♀	10.9	10/29/17	0	67.3			
					½-1			811	
					1		10		
					2	58.6			
					5	72.6			
					7	66.2			
					12		10		
					24	58.8			
					26	45.8			
					29	52.0			Died
XX	Bull terrier	♀	10.0	11/ 1/17	0	51.0			
					0-½			857	
					1		10		
					2	65.0			
					4	71.0			
					6	70.0			
					12		10		Died
XXI	Brown cur	♂	16.8	11/ 2/17	12-24				
					0	60.0			
					½-1			867	
					1		10		
					2	61.0			
					6	64.0			
XXII	Black and white cur	♂	19.3	11/ 6/17	12		10		Died
					12-24				
					0	50.0			
					½-1			851	
					1		10		
					2	50.0			
					5	53.0			
					8	55.0			
					11	50.0			
					12		10		
					14	50.0			Chloroformed

Two gassed dogs (protocols XXIII and XXIV) exposed to this temperature exhibited a prompt fall in the alkali reserve in spite of the injection of morphine in each case. An untreated dog (XXV) exhibited a similar decrease, all three animals dying within three hours after the gassing. A control animal, not gassed, showed at 33°C. external temperature, a fall in alkali reserve (XXVI).

An external temperature of 33°C. appears, therefore, to exert an unfavorable influence upon the alkali reserve of morphinized animals. This series is, however, too limited to permit of any definite conclusions.

NUMBER	DOG			DATE	TIME	VOLUME PER CENT CO ₂ AT 0°	MORPHINE	CHLORINE	FATE
	Kind	Sex	Weight						
			kgm.		hours		mgm. per kgm.	p.p. m.	
XXIII	Cur	♂	12.3	12/4/17	0	48.5			
					$\frac{1}{2}$ -1			891	
					1		10		
					3	46.7			
XXIV	Bull terrier	♂	13.7	12/5/17	4				Died
					0	55.9			
					$\frac{1}{2}$ -1			888	
					1		10		
XXV	Irish terrier	♂	11.4	12/7/17	3	43.8			Died
					5				
					0	52.8			
					$\frac{1}{2}$ -1			858	
XXVI	Black and tan terrier	♂	9.1	12/6/17	3	42.6			Died
					5				
					0	56.0			
					3	50.0			
					5	51.0			

CONCLUSIONS

1. Morphine administered subcutaneously to normal dogs in doses of 10 mgm. per kilo, of the sulphate, causes an increase in the alkali reserve which is maintained at a high level for many hours.

2. Concentrations of approximately 0.08 per cent by volume of chlorine gas produce within from two to seven hours a rapidly advancing condition of acidosis. This may be preceded by a temporary increase in the alkali reserve.

3. In dogs gassed with this concentration of chlorine, morphine, in the 10 mgm. per kilo doses, may temporarily prolong the maintenance of a high alkali reserve level; these doses, however, exert, if anything, an unfavorable influence upon the ultimate outcome.

4. An external temperature of 33°C. appears to affect unfavorably the course of the alkali reserve in morphine-treated gassed dogs.

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ACTION OF ADRENALIN ON THE SPLEEN

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It is generally agreed by recent investigators (1) that adrenalin causes dilatation of the blood vessels in certain parts of the organism. It has been found from experiments carried out in this laboratory that this dilatation is caused, at least in part, by the action upon sympathetic and dorsal root ganglia (2). It may also be caused as shown by Gruber (3) and confirmed by us (4) that the dilatation may result from the stimulation of some peripheral tissue, perhaps myoneural junctions of dilator fibres. It is not possible to say which is more important in producing dilatation normally, the "gangliar" mechanism or the myoneural junction. In some animals the same amount of dilatation has been obtained by the action of adrenalin upon the gangliar portion of the mechanism alone (limb perfused, adrenalin injected into the jugular vein) as occurred from the injection of the same quantity when the circulation was intact (4).

Cats and dogs have been used principally for adrenalin experiments, but work recently published from this laboratory (5) has shown this reaction to be common to Marsupials, Ungulates, and Primates, Rodents being an exception. Therefore physiologists can no longer dismiss adrenalin vasodilatation as an interesting exception.

A careful study has been made of the "gangliar-terminal" action in the hind limb (4), but in many other organs this has not been done. It is the purpose of this and succeeding researches to make a further study of this question in various organs. The present paper is confined to the spleen.

METHODS

Volume changes in the spleen were recorded by enclosing the organ in a gutta percha oncometer connected to a bellows of the Brodie type. The flexible part of the latter was made of rubber cut from a condom. This was fastened to the edge of the bellows base and top with thin glue except at the back where the hinge is located, and where the overlap occurs rubber cement must be used, because the glue when dry stiffens the rubber. Formerly rubber cement was used throughout, but the curling which it causes renders the bellows very difficult to make. The lever for the writing point was attached at right angles to the top of the bellows.

The nerves and blood vessels were carefully freed from fat and connective tissue and then grouped so as to form a double stalk. In many cases it was possible to do this without tying any blood vessels. The spleen was placed in a double-necked oncometer (1e) which was covered with a glass plate connected to the transmission tube.

For perfusion we used one of the large arteries which supplied about one-half of the spleen, the remaining arteries being tied off. Warmed oxygenated Ringer's solution was perfused under a constant pressure produced by compressed air. The temperature and pressure of the perfusion fluid were registered at the entrance to the cannula.

Ether was used as the anaesthetic. Adrenalin solutions were made by diluting Parke, Davis and Company's adrenalin chloride solution with distilled water.

RESULTS

In an earlier research (1e) we made a careful study of the normal spleen in its reaction to adrenalin injected intravenously; seven dogs gave nothing but constriction, while three others responded by dilatation or dilatation and constriction. There seemed to be some question as to the occurrence of active dilatation in the spleen, because in two of the animals, the dilatation preceded the constriction and therefore might be a passive



FIG. 1. WAVES PRODUCED IN A PRACTICALLY QUIESCENT SPLEEN BY THE INJECTION OF A DEPRESSOR DOSE OF ADRENALIN, 0.2 CC., 1:100,000. CAT 2.5 KG.

effect due to constriction elsewhere. Dilatation followed constriction in only one spleen and that was after a large dose of adrenalin.

We have studied four more normal spleens, two of the dog, two of the cat; all but one cat gave dilatation with some dose of adrenalin. In this animal (2.5 kgm.) no dilatation could be secured from a range of doses starting with 0.1 cc., 1:100,000 adrenalin and running as high as 0.5 cc., 1:10,000, however after many of the injections the amplitude of the splenic waves



FIG. 2. WAVES PRODUCED IN A QUIESCENT SPLEEN BY THE INJECTION OF A PRESSOR DOSE OF ADRENALIN, 0.5 CC., 1:10,000. CAT 2.5 KG.

was increased, although the initial effect might be a partial inhibition of the waves. Again the splenic waves might be practically absent until adrenalin was injected, after which they became very marked (fig. 1). Even pressor doses produced a similar effect (fig. 2).

In a second cat (3.7 kgm.) slight dilatation always preceded the constriction which in many cases was followed by waves. Small doses such as 0.1 cc., to 0.5 cc., 1:10,000,000 caused dilatation only.

Although dilatation usually preceded constriction in the two dogs, it occasionally followed the constriction in one animal.

It seemed possible that dilatation of the spleen as in the intestine might be caused by stimulation of the ganglia supplying it. In order to find out whether structures not located in the spleen, could be the cause of the dilatation, we tied all of the blood vessels, and then perfused a portion of it through one of the largest arteries, the outflow being from a vein which had been cut open. Great care was taken to preserve the nerve supply. In this way the effects of adrenalin could be observe either solely upon structures in the spleen by injection into the perfusion fluid or upon structures located outside of the spleen by injection into the general circulation.

We perfused three spleens. The first belonged to a dog weighing 22 kgm. and gave the following responses:

DOSE	PLACE OF INJECTION	RESPONSE IN BLOOD PRESSURE IN MM. OF MERCURY	RESPONSE OF THE SPLEEN
1.0 cc. 1: 100,000	Jugular vein	180-186-158	Dilatation
3.0 cc. 1: 100,000	Jugular vein	177-190-154	Dilatation
5.0 cc. 1: 100,000	Jugular vein	188-194-164	Marked dilatation
1.0 cc. 1: 10,000	Jugular vein	188-210-168	Marked dilatation
0.2 cc. 1: 1,000,000	Through perfusion fluid	None	Constriction, very marked
0.1 cc. 1: 1,000,000	Through perfusion fluid	None	Constriction, marked
0.1 cc. 1: 10,000,000	Through perfusion fluid	None	No effect

The perfused spleen of this animal dilated with every dose of adrenalin injected into the jugular vein (fig. 3), but constricted with each injection into the perfusion fluid (fig. 4). The latter was injected into the fluid just before it entered the cannula. The pressure for perfusion was 45 mm. of mercury while the temperature was 33.4°C.

The second spleen did not seem to be responding very well and no effect could be obtained except a slight constriction when adrenalin was introduced into the general circulation.

The third perfused spleen, belonging to a dog weighing 10 kgm., dilated considerably with the doses of adrenalin injected

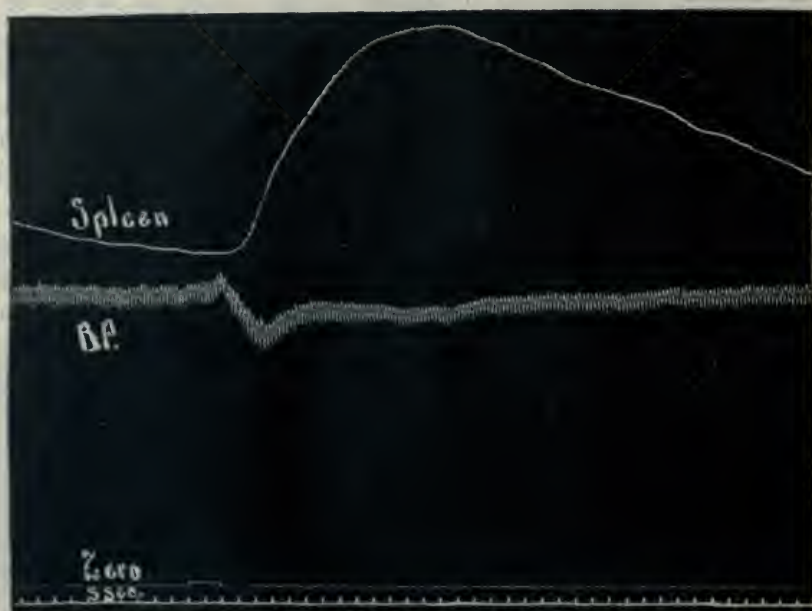


FIG. 3. DILATATION OF A PERFUSED SPLEEN FROM THE INJECTION OF 5.0 CC. 1:100,000 ADRENALIN INTO THE JUGULAR VEIN. DOG 22 KGM.

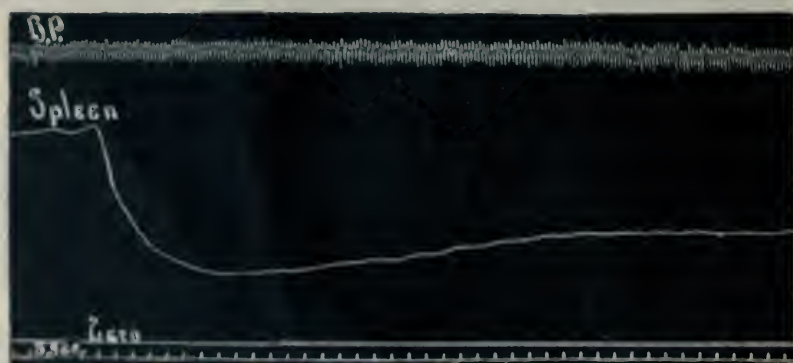


FIG. 4. CONSTRICTION OF A PERFUSED SPLEEN FROM THE INJECTION OF 0.1 CC., 1:1,000,000 ADRENALIN INTO THE PERFUSION FLUID. DOG 22 KGM.

into the jugular vein, 0.5 cc., 1:100,000 to 0.5 cc., 1:10,000. These doses were depressor in their effect upon the blood pressure. When adrenalin was injected into the perfusion fluid, constriction was followed by dilatation (fig. 5). This was true even with a relatively large dose, 0.2 cc. 1:100,000. The dilatation, however, was not as marked as that produced from the mechanisms outside of the spleen.

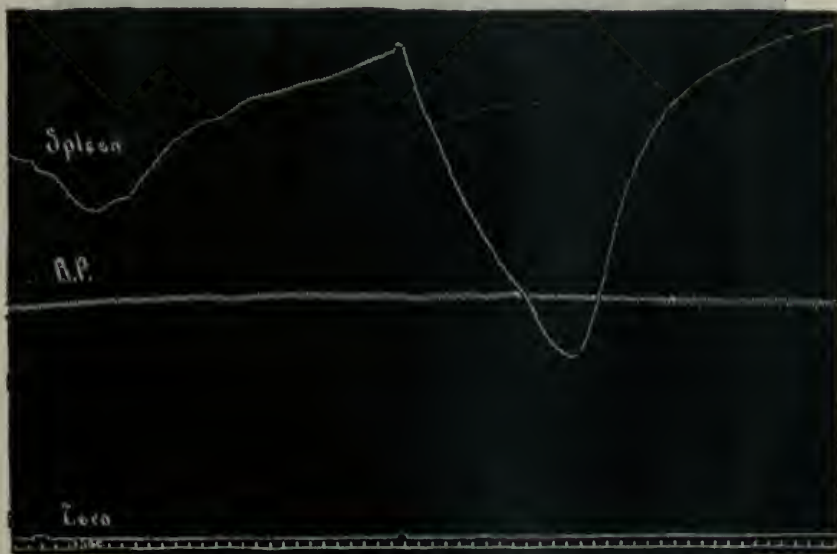


FIG. 5. CONSTRICTION FOLLOWED BY DILATATION, PRODUCED BY THE INJECTION OF ADRENALIN INTO THE PERFUSION FLUID ENTERING A PERFUSED SPLEEN.

First injection 0.2 cc., 1:1,000,000; second injection, 0.2 cc., 1:100,000. Dog 10 kgm.

In order to determine whether the semilunar ganglion contained mechanisms which might cause dilatation of the spleen through the action of adrenalin, direct application of adrenalin to this ganglion was tried while the spleen was in an oncometer with its circulation intact. If no changes in blood pressure occurred during the experiment we were justified in assuming that adrenalin was not passing into the blood stream and there-

fore could produce its effect only by gangliar action. Absorption was facilitated by slitting the surface of the ganglion.

The spleen of a cat was studied by this method. Solutions of 1:100,000 were twice applied without changing the blood pressure, but in each instance causing dilatation of the spleen. A third application of a stronger solution caused a very marked dilatation (fig. 6).

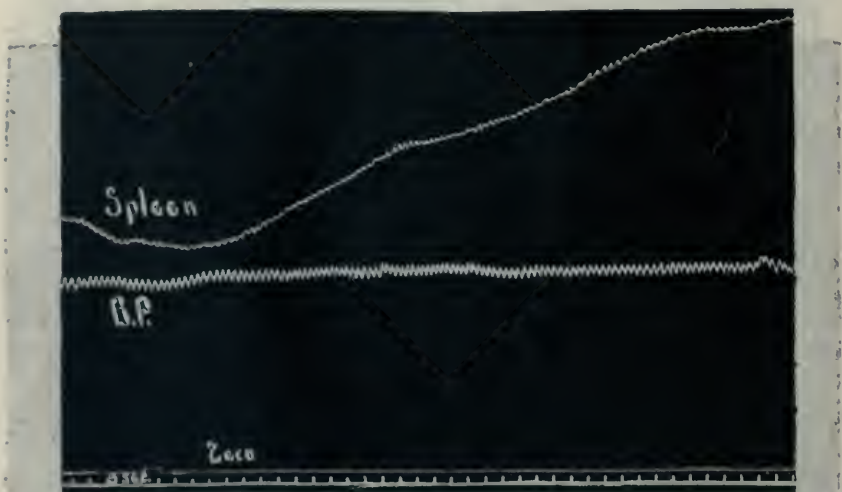


FIG. 6. DILATATION OF THE SPLEEN CAUSED BY THE DIRECT APPLICATION OF 1:10,000 ADRENALIN TO THE SEMILUNAR GANGLION. CAT.

This experiment therefore demonstrates that the semilunar ganglion is one location of the adrenalin dilator mechanism of the spleen.

We also studied the effect of adrenalin upon the spleen through action upon the ganglia of the dorsal nerve roots. These ganglia were exposed and painted with solutions of adrenalin after cutting the connections with the spinal cord. In some instances the ganglia were split open to facilitate absorption. Blood pressure records were taken at the same time. Care must be taken not to stimulate the ganglia mechanically, for sometimes that will cause splenic volume changes. The twelfth and thirteenth thoracic ganglia on the left side were almost always used.

Five cats were studied. All showed some response to adrenalin applied to the above ganglia, although in some instances only one response could be obtained from a single ganglion, a new ganglion being required in that case to secure a repetition of the response. The volume changes are usually slow in occurring and likewise slow in disappearing unless the ganglion is washed to remove the adrenalin.

One animal responded by dilatation only with concentrations of 1:10,000 and 1:1000; weaker solutions were not tried.

Three of them gave dilatation sometimes and constriction at other times, there being no regularity in the occurrence of either.

The fifth cat was interesting in that adrenalin applied to the ganglia in question, caused waves in the spleen if it were quiescent, or increased the amplitude of the waves if they were already present.

We would conclude from our observations that both constrictor and dilator mechanisms for the spleen are present in the dorsal root ganglia. We cannot say which predominates.

DISCUSSION

Oliver and Schäfer (6) were the first to study the action of adrenalin (adrenal extract) upon the spleen. In no cases did they obtain a dilatation except "a very slight preliminary expansion," probably caused by the increased heart's action. A later paper by Schäfer and Moore (7) added to this observation that the after effect of the injection was to increase the extent of the normal rhythmic movements. When injected into a perfused spleen a strong contraction was obtained.

Bardier and Fränkel (8) obtained dilatation from macerated adrenals. Others (9) speak only of contraction of the spleen from adrenalin.

Recently Hoskins and Gunning (1c) and Hartman and McPhedran (1e) have observed mainly constriction from adrenalin in the spleen. The former speak of a brief dilatation followed by contraction. They occasionally obtained an active dilatation following the constriction.

Our experiments have proven that dilatation from adrenalin can be obtained by action upon structures in the semilunar ganglion, and the dorsal root ganglia as well as by action upon structures in the spleen. In this respect the adrenalin dilator mechanism of the spleen is similar to that of skeletal muscle (as shown by the hind limb, 4).

There can now be no doubt that adrenalin produces active dilatation of the spleen. Judging from our experiments the gangliar mechanism gives the dilator effects more easily than does the peripheral mechanism. That may be due to a partial masking of the dilator mechanism by the constrictor mechanism in the latter region.

In regard to the peripheral effect there seems to be a distinct difference between the limb reaction and that of the spleen. In the perfused limb small amounts of adrenalin injected into the perfusion fluid cause pure dilatation, larger amounts may cause dilatation followed by constriction, while very large doses may cause pure constriction. On the other hand in the perfused spleen if dilatation is obtainable from adrenalin injected into the perfusion fluid, it follows constriction, at least in our experience.

SUMMARY

1. Dilatation of the spleen is caused by the action of adrenalin upon the twelfth and thirteenth dorsal root ganglia, the semilunar ganglion or upon some terminal structure in the spleen itself.
2. Constriction from adrenalin can result from the response of a mechanism in the dorsal root ganglia or from a structure in the spleen.

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COMPARATIVE ACTIVITY OF LOCAL ANESTHETICS

VI. DIFFICULTLY SOLUBLE ANESTHETICS ON MUCOUS MEMBRANES¹

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METHODS

The efficiency of the difficultly soluble anesthetics for mucous membranes may be estimated, although somewhat roughly, on the cornea of rabbits, or on the gums of human subjects. In either case, the threshold values are compared by using dilutions made with powdered talcum. Neither method is altogether satisfactory, but they check each other quite well, and should show marked differences in efficiency, if such exist.

CORNEAL METHOD

In making the tests, the rabbits are placed in snugly fitting individual stalls. The lashes are clipped. The winking reflex is tested by touching the cornea with a pointed pencil. The anesthetic, diluted to the desired degree with talcum, is then applied with a simple powder blower. This consists of a glass cannula attached to a rubber syringe-bulb. The tip of the cannula is filled by pressing it into the anesthetic powder, and this is then blown on the cornea. The sensitivity is tested after five minutes, repeating the applications until the cornea can be touched without winking, or, if the powder is ineffective,

¹ The investigation was supported by a grant from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

until six applications have been made. If anesthesia occurs, the observations are continued, at five minute intervals, until sensation returns.

Results of the corneal method. These are shown in table 1. I am indebted to Miss J. R. Collacott for the observations. A indicates the number of applications (with five minute intervals) required to produce anesthesia, "nil" meaning that anesthesia did not result after six applications. R. signifies the minutes elapsing between the last application, and complete recovery of sensation. In the observations marked *, the nature and percentage of the anesthetic were unknown to the operator.

TABLE 1

Insoluble anesthetics on rabbits' cornea. The symbols are explained in the text

PERCENTAGE	ANESTHESIN		CYCLOFORM		ORTHOFORM-NEW		PROPAESIN	
	A	R	A	R	A	R	A	R
100.0					3; 3	25; 20		
75.0					3	25		
50.0	3	15	4	15	4	30	2	30
35.0					3	20		
25.0	3	25	5	10	3	20	3	20
10.0	3; 4*	15; 10	5; 3*	10; 15	2	15	3	15
5.0	4*; nil	10	3*; nil	10	nil; nil		5; nil	5
2.5	nil	nil			nil; nil		5; nil	5

APPLICATION TO THE GUMS

The powder is applied with a spatula to the outside of the gums, and well into the sulcus of the lip. The observations were made on one subject, at the left second incisor. Usually only one substance was tried each day. The sensibility was tested with a pointed pencil. The applications were renewed every two or three minutes, until the maximum effect had been reached, or until some six applications had proved ineffective. The inaccuracies of the method arise mainly from the rather poor sensitiveness of the normal gums.

Results of applications to the gums. These are shown in table 2.

TABLE 2

Anesthesia from insoluble anesthetics on the gums

PERCENTAGE	ANESTHESIN	CYCLOFORM	ORTHOFORM-NEW	PROPAESIN
25.0		Almost complete (6)	Almost complete (2)	
10.0	Almost complete (4)	Slight (5) Almost complete (3)	None (6) Doubtful	Almost complete (4)
5.0	Almost complete (4) Doubtful (5)	Almost complete (2)	Slight (4)	Considerable (3)
2.5	Very slight (6)	Almost complete (3)	Doubtful (4)	Slight (4)

The numbers in parentheses are the numbers of applications required to produce the maximal effect.

CONCLUSIONS

The efficiency of the difficultly soluble local anesthetics for mucous membranes may be demonstrated on the cornea of rabbits, or on the gums of human subjects. An approximate estimate of their relative efficiency may be obtained by comparing dilutions made with talcum powder.

On the cornea, anesthesin, cycloform, orthoform-new and propaesin appear to be practically equivalent.

They are certainly effective in concentrations of 10 per cent; uncertain at 5 per cent, and practically ineffective at 2.5 per cent.

The anesthesia persists for ten to fifteen minutes with 10 per cent; fifteen to thirty minutes with 25 to 100 per cent.

On the gums, a greater variation was noted. The minimum concentrations giving almost complete anesthesia were as follows:

	per cent
Cycloform less than.....	2.5
Anesthesin.....	2.5 to 5.0
Propaesin.....	5.0 to 10.0
Orthoform new.....	10.0 to 25.0

A FURTHER CONTRIBUTION TO THE PHARMACOLOGY OF THE LOCAL ANESTHETICS¹

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The search for substitutes for cocain has led to the introduction into the materia medica of a large number of compounds possessing the property of causing local anesthesia, and for each of these the claim has been made that it is less toxic than cocain. These compounds belong to several general chemical groups. As we shall show later, not only do these compounds of somewhat diverse chemical nature possess the common property of producing local anesthesia, but also they resemble one another very closely in all their more important pharmacologic actions, such differences as are shown being chiefly quantitative. That they are all capable of producing severe, or even fatal, acute poisoning in man, with symptoms closely analogous to those produced by cocain, will also be seen in the brief review of the more important literature which we give before passing to the presentation of our experiments.

REVIEW OF THE LITERATURE

The occurrence and symptoms of acute cocain poisoning are so well known that it is unnecessary to do more than refer to one or two articles. Mattison (1) presents in abstract a large number of acute fatal and non-fatal cases of cocain poisoning; and a good bibliography of cocain poisoning is to be found in a paper

¹ Part of the expense of this research has been defrayed by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

by Wildenrath (2). In a previous paper we (3) cited the fact that death has been recorded following such small doses of cocain as 16, 40 and 60 mgm., respectively, while 1.25 grams, injected subcutaneously, have been survived. The typical symptoms and course of acute, non-fatal intoxication are described by Lodge (4). The patient, a man forty years old and in good health, received a subcutaneous injection of 20 minims of a 2 per cent solution of cocain—about 13 mgm. of the drug. In two to three minutes the patient became nervous with heightened reflexes, rapid pulse, deepened respiration, vertigo, and flickering before his eyes. Nausea developed in about ten minutes, followed by vomiting. At the end of about an hour after the injection the toxic symptoms were at their height, when the following were noted:

Pulse 120, respirations deepened, the inspiratory time probably increased to four times the length of the expiratory time; reflexes greatly increased, clonic spasms of all the muscles of the limbs, arms and inferior maxillary . . . pupils markedly dilated . . . muscles of the lower jaw were in tonic contraction.

The patient recovered in about two hours after the injection, but he did not sleep well that night, and had a severe headache the next day. This case illustrates the promptness of beginning absorption and the rather prolonged action of the cocain—both facts of importance, as will be shown subsequently.

The literature of tropacocain is comparatively meagre, and we have not found any record of fatal intoxication in man from the use of this drug. We venture to express it as our belief, however, that this drug is little, if any, less toxic for man than is cocain, though it is possible that man may not show the same idiosyncrasy toward it as he does toward cocain.

Marcinowski (5), in an extensive paper on the clinical uses of beta-eucain, comments on its low toxicity for man. He says that cocain is 3.75 times as toxic for animals as beta-eucain, but he does not mention the species, the mode of administration, or the concentration of the solutions, hence his figures are virtually worthless. He gives a bibliography of 202 papers, chiefly clin-

ical, and he goes so far as to state that where beta-eucain can be substituted for cocain it is little short of criminal negligence not to do so. He also makes the direct statement that in man general intoxication has been seen only after the use of beta-eucain in the spinal canal.

Kraus (6) records a case of severe acute poisoning from the injection into the urethra of a healthy man of 0.2 gram of beta-eucain in 2 per cent solution. The day preceding, the same dose had been similarly injected for the incision of a stricture, and had produced no symptoms whatever. He believes that the acute intoxication resulted from the rapid absorption of the drug through the recent wound.

Way (7) also records a case of serious intoxication from the infiltration about the genitals for circumcision of 0.12 gram of beta-eucain in a solution containing a few drops of epinephrin. In both this and the preceding case the symptoms appeared promptly after the injection, and in both clonic or tetanic convulsions were noted. Recovery was apparently more rapid in Way's patient than is seen after cocain, while Kraus' patient still had some symptoms after the lapse of nearly two hours.

The more important literature of intoxication by stovain has been reviewed in a paper by Smith and Hatcher (8), to which the reader is referred. It is well known that many cases of serious, or fatal, intoxication have resulted from the use of this drug, especially for intraspinal anesthesia.

Alypin was introduced into the materia medica by Impens and Hofmann with claims of superiority over cocain and the cocain substitutes, and Impens (9) presents an extensive pharmacologic study of its actions and toxicity, in which he states that for cats and dogs alypin is only half as toxic as cocain by subcutaneous injection. He concludes from his experiments, that the danger from alypin in man must also be much less than that of cocain. He, however, shows clearly that the toxic actions of alypin and cocain are essentially the same qualitatively in the lower animals, although he contends that there is less tendency to variations in the responses of different animals to a given dose of alypin than to cocain.

That severe intoxication does occur with alypin is seen by the following references to recorded cases. Garasch (10) records two cases of severe poisoning resulting from the intraurethral instillation of 5 cc. of a 5 per cent solution, and 5 cc. of a 2 per cent solution, respectively (0.25 and 0.1 gram, respectively) in two young men for the purpose of instrumentation. The symptoms began in one and a half to two minutes, rapidly reached their maximum, and both patients had fairly completely recovered within about twenty minutes. In both instances the heart and respiration had stopped, or nearly stopped, and convulsions were repeated. The author says that these are the only cases of severe intoxication from alypin in his total of 1453 cases of its use in genito-urinary surgery. He also records the injection into the bladder of an old man with carcinoma of that viscus of 15 cc. of a 10 per cent solution (1.5 grams) of alypin without the production of any symptoms.²

Ritter (11) records an instance of acute intoxication resulting in death, following the infiltration into the region of the thyroid gland of about 1 gram of alypin in 2 per cent solution. Although the initial symptoms were typical of acute alypin poisoning, it is not certain that death was wholly due to that drug, as the patient was suffering from obstructed breathing due to a goiter, and had also received morphin and adalin before the infiltration.

Other cases of severe, or fatal, intoxication have been reported; thus Proskauer (12) records sudden death after injection into the urethra of 20 cc. of 2 per cent solution of alypin (0.4 gram) following injury to the mucosa by attempted passage of a cystoscope; and Schroeder (13) records two non-fatal cases following intranasal infiltration of 13 cc. and 18 cc., respectively, of a 2 per cent solution of alypin (0.26 and 0.36 gram), followed by application by spray and tampons of a 15 per cent solution in small amounts. Both of the patients had previously received 0.6 mgm. of scopolamin. In both patients the symptoms of intoxication began in less than ten minutes after the

² Translated by Mr. Solomon Krell, technical assistant.

injection of the alypin, and both recovered promptly after artificial respiration and the administration of cardiac stimulants. Jacobs (14) records two non-fatal cases following the intra-urethral instillation of 2 per cent alypin solution, both patients having sustained injury to the urethra by previous effort to pass an instrument. Each of these patients received a dose of about 0.16 gram of alypin, and although both had violent clonic convulsions and stopped breathing, recovery was very rapid following artificial respiration.

We have not found any references to severe acute poisoning by either holocain or nirvanin, but this is probably due to the fact that neither of these drugs has been extensively employed in clinical practice. Certainly it is not due to their slighter toxicity.

The question of accidental poisoning by procain (novocain) has been reviewed in our earlier paper and does not require further mention, except that we wish to emphasize the fact that in the non-fatal cases recovery from the threatening symptoms has generally been rapid. Following the publication of that paper a plea for the reporting of all cases of accidents from the use of local anesthetics was published broadcast in the medical, dental and pharmaceutical journals, signed by Torald Sollmann, chairman of the Therapeutic Research Committee of the Council on Pharmacy and Chemistry, and by Robert A. Hatcher. Although more than a year has elapsed since that plea must have reached every physician, dentist and pharmacist who reads his journals, only four cases of procain, and five of cocain, poisoning have come to our attention, all privately reported. In but two of these cases were the details given sufficient to warrant their mention. One was a case of severe intoxication in a woman thirty years old and on the verge of a nervous breakdown. Forty minims of 2 per cent solution of procain (about 50 mgm.), containing 1-50,000 epinephrin, were injected into the mandibular foramen and less than eight minutes later the patient developed clonic convulsions and slowing of the pulse. Camphorated oil and aromatic spirit of ammonia were administered and recovery was complete in less than two hours. The second case resulted

from the massage into the nose of about 65 mgm. of cocain in a solution containing 10 drops of 1-1000 epinephrin. The patient promptly had three seizures of clonic convulsions with great weakening of the heart but recovered in half an hour, aromatic spirit of ammonia having been employed as a stimulant.

Two facts seem to stand out clearly from the preceding review:

1. That moderate doses of cocain and its substitutes are capable of producing severe, or fatal, acute intoxication in man under a variety of conditions, both with respect to the patient and to the mode of application of the drug.

2. That the rapidity of absorption of the drug into the blood stream seems to be the factor of greatest importance in determining the occurrence of acute intoxication, especially in the case of the several substitutes for cocain.

It will be admitted by all that it would be of the greatest value to determine accurately the several factors which lead to the production of such acute intoxications; their relative importance; their relative frequency of occurrence; and the methods of anticipating them or of avoiding them. But, while animal experiments may throw some light on these problems, their accurate solution can come only from the collection and careful study of clinical accidents. It is, therefore, of first importance that everyone who encounters a case of accidental intoxication should report his observations with as great accuracy and detail as he can. It is no reflection on the physician or dentist that he should occasionally meet with accidental poisoning from these drugs, but rather is it a reflection that he should be unwilling to publish his own experiences in their fullest detail and aid in the future avoidance of similar accidents.

EXPERIMENTAL

The present investigation was undertaken in the attempt to throw some light upon the causes of acute intoxication in man produced by the several local anesthetics; to discover, if possible, some of the factors which influence the toxicity of these drugs; and to find means of avoiding, or combating, their acute toxic

actions. We have therefore made observations on the following points:

1. The phenomena of acute intoxication after intravenous injection.
2. The maximum toxicity by rapid intravenous injection.
3. The mutual synergism of the several local anesthetics.
4. The toxicity by slow or repeated intravenous injections.
5. The toxicity by subcutaneous injection.
6. The influence of epinephrin on toxicity by subcutaneous injection.
7. Notes on absorption from mucous membranes.
8. Elimination by the liver.
9. Efforts to influence the toxicity of the local anesthetics.
10. Synergism with epinephrin on systemic administration.
11. Measures for resuscitation.
12. Antagonism by ouabain.

More than three hundred experiments were performed; hence we will present only a few typical protocols and give the general results in the form of tables and discussions. We have also drawn upon the results of previous work in our laboratory. All of the experiments were made upon cats, which more closely resemble man in their responses to most drugs than do the rodents, especially with reference to absorption from the subcutaneous tissues. Dogs were not used because, owing to their larger size, we would not have had sufficient supplies of most of the drugs to carry out the investigations planned, and further supplies were unobtainable; and also because we had previously shown that these animals behave essentially as do cats to the local anesthetics. We omitted the use of rabbits, in part because of their almost prohibitive expense, and because it has been shown that they do not differ essentially from cats in their susceptibility to these drugs by rapid intravenous injection (Schroeder; Hatcher and Eggleston; Smith and Hatcher).

The following local anesthetics were employed, the hydrochlorids being used throughout:³ alypin, apothessin, beta-eucain,

³ We are indebted to Prof. Torald Sollmann for having generously provided us with supplies of several of the synthetic cocain substitutes at a time when these were not commercially available.

cocain, holocain, nirvanin, procain (novocain), stovain, tropacocain.

Except where otherwise indicated, all operative procedures were carried out without the use of a general anesthetic. Where it was necessary to expose a vein or an artery the hair was clipped closely and a 10 per cent solution of phenol in oil was used as a local anesthetic. In all experiments, except where stated to the contrary, the local anesthetic was dissolved in 0.85 per cent sodium chlorid solution. In every instance the solutions were prepared on the day on which they were to be used, except in the case of tropacocain, which was sometimes kept in solution for two or three days.

1. Phenomena of acute intoxication after intravenous injection

When a severely toxic, but not fatal, dose is rapidly injected into the femoral vein the general symptoms produced are essentially the same for all of the local anesthetics studied. Within a few seconds there are more or less pronounced stimulation of the respiration and an abrupt fall of blood pressure, the heart rate varying, but usually being slowed. The respiration may then stop for several seconds, or may continue to show stimulation, both rate and depth being increased. Within about a minute the heart begins to improve and the blood pressure rises; at about the same time the respiratory stimulation begins to pass off, and commonly at about this time there is one, or several, brief but severe clonic convulsions. Within less than five minutes the animal is apparently quite normal. The fall in blood pressure is due to a weakening of the heart and not to any immediate vasodilatation. The length of time required for the onset of symptoms does not differ materially for the different drugs or for doses of different size, so long as the dose is a toxic one. There is, however, some difference noted for each of these factors in the rate of recovery. After doses just sufficient to produce the phenomena described complete recovery seldom takes over five minutes, but when the doses are nearly fatal the time of recovery may be about doubled, repeated clonic con-

vulsions being continued for from six to ten minutes after the injection. The different drugs, also, may be divided into two groups with reference to the rate of recovery following a single sublethal dose. Cocain and holocain are the only members of the first group, and are characterized by a decidedly more prolonged action than is seen after any of the other cocain substitutes. This difference will be more clearly shown when we

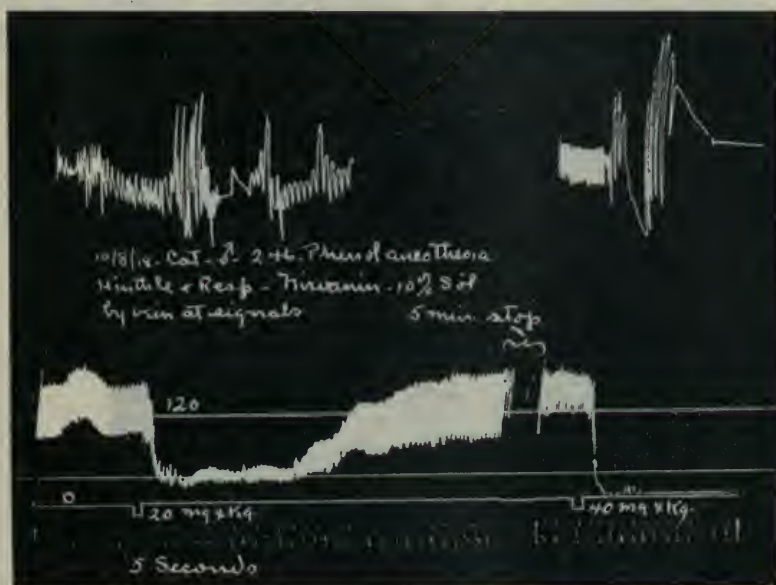


FIG. 1

come to the discussion of repeated doses, and it is one of prime importance. The second group comprises all the other drugs studied; which show only slight differences in the rates of recovery, and from all of which recovery is rapid.

The effects upon the heart and circulation, upon the respiration, and the rapid recovery from these effects, are shown in the tracing—figure 1 which accompanies the following protocol.

Protocol 1. Cat, male, 2.46 kgm.

Twenty milligrams x kgm. nirvanin in 10 per cent solution at once into femoral vein. Sharp fall of blood pressure in less than ten seconds, followed at once by respiratory stimulation. Then respiration almost stopped, while heart began to show slight recovery. Within two minutes after the injection the blood pressure had returned to normal. Clonic convulsions interfered with the respiratory tracing.

Recovery was complete in less than ten minutes; when a surely fatal dose was given and followed by prompt cessation of the heart, transient respiratory stimulation and death.

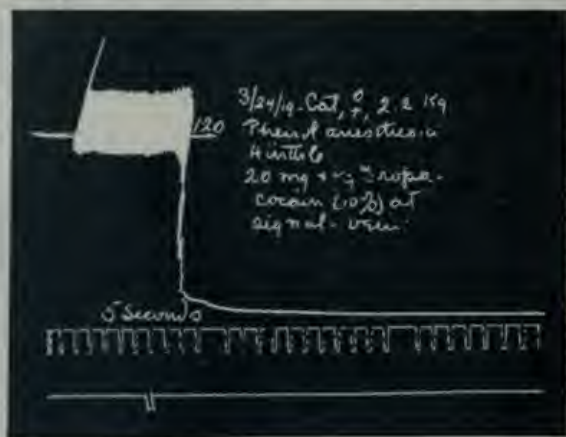


FIG. 2

When fatal doses are given there is often an almost instantaneous general extensor spasm, combined with fixation of the respiration and sudden cessation of the heart beat. After an interval of less than thirty seconds the respiration usually returns in the form of a few shallow gasps, while the heart may resume beating feebly and slowly and continue for a few seconds to several minutes. At other times the auricles alone continue to beat for several minutes, the ventricles not participating, as shown by arterial tracings or by direct inspection of the heart. The abruptness with which the heart may be brought to a standstill is shown in the tracing—figure 2—accompanying the following protocol.

Protocol 2. Cat, female, 2.2 kgm.

Twenty milligrams x kgm. tropacocain in 10 per cent solution at once into femoral vein. Almost instantaneous, violent extensor spasm, respiration fixed; heart stopped suddenly in ten seconds. Few respiratory gasps. Death.

The outstanding features of the phenomena of the toxic actions of all of the local anesthetics, when injected rapidly into the circulation are:

1. The rapidity of onset of the effects upon the heart and respiration.

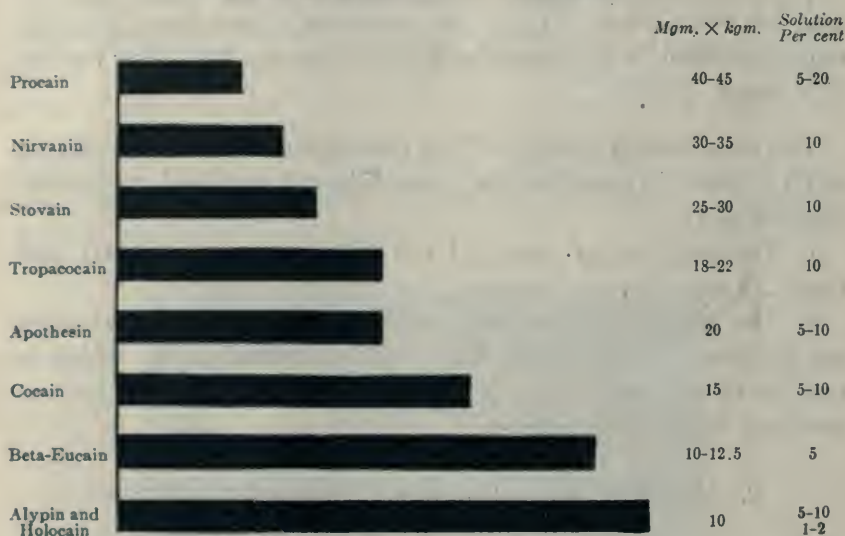
2. The rapidity of complete recovery, even after doses which are sufficient to stop both the heart and respiration, which is true of all of the drugs studied, except cocain, with which the recovery is decidedly slower.

2. Maximum toxicity by rapid intravenous injection

The toxic actions of the local anesthetics being exerted only after the entrance of the drugs into the circulation, we sought to determine the approximate maximum toxicity of each after very rapid intravenous injection of concentrated solutions. We found that it made little difference whether the solution employed was 5 per cent or 20 per cent, since within those limits the dose could be injected from a syringe in from five to fifteen seconds. For the sake of accuracy, however, we append the concentrations employed for each of the drugs. Chart 1 presents in graphic form the relative toxicities of the nine compounds studied; gives the minimal fatal dose in milligrams per kilogram; and shows the range of strengths of the solutions employed. We have taken our figures for procain and stovain in part from our previous papers.

In all cases death is apparently due to the essentially simultaneous paralysis of the heart and respiratory center. In some cases the heart may return and beat feebly for a few seconds after the respiration has failed (see fig. 3), while in others there may be some furtive efforts at respiration after the heart has

CHART 1. SHOWING RELATIVE TOXICITY; FATAL DOSES IN MILLIGRAMS PER KILOGRAM; AND RANGE OF CONCENTRATION OF SOLUTIONS FOR THE SEVERAL LOCAL ANESTHETICS



stopped. The direct action on the heart is shown in a tracing taken from a perfused heart by Smith and Hatcher, and in the immediate stoppage of the heart recorded in some of the tracings we reproduce here.

From the chart it is seen that cocain occupies an intermediate position with reference to its maximal toxicity, being surpassed by alypin, beta-eucain and holocain, while the least toxic are nirvanin and procain. It is interesting to note that alypin, which is chemically closely related to stovain, is about three times as toxic, while cocain and tropacocain, which are very closely related, differ appreciably in toxicity, cocain being a third more toxic than tropacocain.

3. *Mutual synergism of the several local anesthetics*

The striking similarity noted between the systemic actions of all of these local anesthetics, especially upon the heart and respiratory center, led us to conduct a series of experiments to determine whether or not they are synergistic, and if so to



FIG. 3

what extent. The accompanying protocols illustrate our methods of procedure and the results obtained.

Protocol 3. Cat, male, 3.46 kgm.

Twelve milligrams x kgm. nirvanin (37 per cent of fatal dose) plus 18 mgm. x kgm. procain (43 per cent of fatal dose) injected at once into femoral vein in 10 per cent solutions.

Immediate violent respiratory stimulation and distress; heart did not stop; typical rapid recovery.

The total represented 80 per cent of the combined fatal doses.

Protocol 4. Cat, female, 2.2 kgm.

Five milligrams x kgm. alypin (50 per cent of fatal dose) plus 7.5 mgm. x kgm. cocain (50 per cent of fatal dose) at once into femoral vein, in 10 per cent solutions.

Immediate respiratory stimulation; heart stopped in about ten seconds; respiration stopped in about one and a half minutes.

The total represented 100 per cent of the combined fatal doses.

These two protocols are typical of our results throughout, which showed that all of the local anesthetics studied are mutually and quantitatively synergistic. Thus the administration intravenously of a mixture of any two is followed by the general train of symptoms already described, or the administration of a measured fraction of the fatal dose of one plus such a fraction of any other as will make the two fractions equal to 100 per cent of the combined fatal doses of the two results in death with symptoms which are typical of the whole group. Nothing would be gained by the further multiplication of protocols.

4. Toxicity by slow or repeated intravenous injections

We have emphasized, in the section dealing with the maximum toxicity of the local anesthetics by intravenous injection, the fact that the fatal dose is thrown into the blood stream very rapidly—in fact virtually all at once. We have also mentioned the fact that recovery from a sublethal dose is very rapid, except in the case of cocain, even when the dose approaches closely to the fatal. We have also shown in our earlier paper that many times the minimal fatal dose of procain (then called novocain) can be injected intravenously if the injection be made slowly, or if a series of sublethal doses be given at intervals of about fifteen minutes. The same was also shown to be true of stovain by Smith and Hatcher.

In our paper on procain we gave a protocol showing that cocain could not be given by repeated intravenous injection of small doses without the development of evidences of cumulation, as shown by the increasing severity of the symptoms following the later repetitions, and by the ultimate death of the animal after a total of five times the minimum single fatal dose, given in a period of four hours and sixteen minutes in a series of fifteen injections. We have confirmed this observation and have investigated the remaining drugs in this series in the same way,

as well as by slow and nearly continuous injection of relatively dilute solutions. Before discussing the results of this portion of our work we will present two abbreviated protocols.

Protocol 5. Cat, female, 2.46 kgm.

- 10.17 a.m. 20 mgm. x kgm. nirvanin at once into femoral vein, in 5 per cent solution. Almost immediate convulsion; heart impalpable; enormous respiratory stimulation; followed by prompt recovery.
- 10.32 a.m. 20 mgm. x kgm. nirvanin as above; symptoms as before, but heart did not stop.
- 10.47 a.m. 25 mgm. x kgm. nirvanin as above; symptoms as before.
- 11.07 a.m. 30 mgm. x kgm. nirvanin as above; symptoms as before.
- 11.30 a.m. 40 mgm. x kgm. nirvanin as above; symptoms as before; but heart stopped almost instantly. After half a minute heart began to beat again.
- 11.32 a.m. Animal nearly normal.

Recovery complete.

This animal received 135 mgm. x kgm. of nirvanin—more than three times the single fatal dose—in 1 hour and 13 minutes, the last dose being equal to the minimal fatal dose. Recovery was as prompt after the last dose as after any of the preceding. This indicates that the drug was fixed or destroyed at the rate of one fatal dose every twenty minutes.

Protocol 6. Cat, female, 2.55 kgm.

- 2.32 p.m. Start injection of beta-eucain, 1-1000, into femoral vein.
- 3.05 p.m. 38 cc. total injected; no symptoms.
- 3.25 p.m. 60 cc. total injected; slight convulsive movements.
- 3.40 p.m. 83.7 cc. total injected; no increase in symptoms.
- 3.45 p.m. Animal released; stands, but is somewhat unsteady from long restraint, otherwise seems normal.

In this case a total of 37.5 mgm. x kgm. of beta-eucain, or nearly four times the fatal vein dose, was injected in one hour and eight minutes without producing serious symptoms. The indicated rate of destruction or fixation is at least as rapid as one minimal fatal dose every twenty minutes.

The rates of destruction or elimination for the several drugs investigated seem to be about as follows: Alypin, beta-eucain,

apöthesin, nirvanin, procain, and stovain are destroyed at the rate of at least one fatal vein dose every twenty minutes. Holocain is apparently destroyed a little less rapidly, one fatal dose requiring about half an hour; while cocain is not destroyed more rapidly than about one fatal dose per hour, if as rapidly as that, and the rate of its destruction apparently decreases progressively.

In connection with the elimination of certain of the digitalis bodies we have (15) spoken of the elimination of a poison from those organs upon which its essential, or toxic, actions are exerted as the "essential" elimination to distinguish it from that elimination which may take place immediately after the drug enters the blood stream. In the case of these local anesthetics this "essential" elimination proceeds at a very rapid rate and is accomplished, as we shall show later, by destruction or fixation of the drug. Even in the case of cocain "essential" elimination is rapid at first, although relatively much less so than with the remaining local anesthetics. Unlike the digitalis bodies the "essential" elimination of the local anesthetics is virtually the same as the immediate elimination and is practically identical with total elimination, excepting in the cases of cocain and holocain.

This affords an excellent illustration of the difference between those drugs which show the so called "cumulation" and those which do not. In the case of the former the immediate elimination from the blood may proceed very rapidly, but "essential" elimination is a slow process, so that the administration of repeated doses leads to an increasing amount of the drug which is present and acting upon the essential organs. In the case of the latter the administration of repeated doses finds the organs upon which the essential actions are exerted fully restored to normal and quite free from remaining fractions of the previous dose or doses.

5. Toxicity by subcutaneous injection

The relative toxicity of several of the local anesthetics by subcutaneous injection has been studied by LeBrocq (16);

Chadbourne (17), Grode (18), by Schroeder, and by Impens. We have prepared table 1 from the figures given by these authors for the toxicity in rabbits.

Examination of the various doses stated as fatal for the rabbit after subcutaneous injection of cocain, tropacocain and alypin shows a wide range of variation, the largest dose stated for cocain being more than twice the smallest; the largest for tro-

TABLE 1

Showing the fatal subcutaneous doses for rabbits of alypin, cocain and tropacocain, according to various authors

ANESTHETIC	FATAL DOSE	AUTHOR
	<i>mgm. x kgm.</i>	
Alypin.....	115-147	LeBrocq
	110	Schroeder
	50	Impens
Cocain.....	200	Chadbourne
	153-192	LeBrocq
	100-150	Impens
	87-100	Grode
Tropacocain.....	over 500	Chadbourne
	281-345	LeBrocq

pacocain more than one and three-fourths times as great as the smallest; and the largest for alypin being nearly three times the smallest. Three possible explanations may be offered for such divergence.

1. Variation in the susceptibility of the animals.
2. Variation in the concentrations of the solutions used.
3. Variation in the rates of absorption from the subcutaneous tissues.

We are inclined to believe that in view of the rapidity of the destruction of these drugs the latter is the probable explanation. In fact, our observations on the rate of destruction after intravenous injection led us to abandon the effort to fix accurately the fatal subcutaneous doses for the several local anesthetics. We therefore confined these observations to a few experiments

with each drug to observe the symptoms produced, the approximate rates of absorption and destruction, and to determine whether or not the relation of destruction to absorption was such as to enable the cat to survive at least five times the average fatal vein dose when injected subcutaneously. A few protocols will illustrate the results obtained.

Protocol 7. Cat, female, 3.34 kgm.

- 9.40 a.m. 60 mgm. x kgm. cocain in 10 per cent solution subcutaneously.
- 9.48 a.m. Emesis.
- 9.57 a.m. Clonic convulsions; heart slow and strong; respiration only moderately stimulated . . . convulsions repeated nearly continuously.
- 10.51 a.m. Convulsions over.
- 10.57 a.m. Heart very slow; respiration stops for periods and then returns; is very slow and feeble.
- 11.07 a.m. Respiration stopped.
- 11.10 a.m. Heart stopped.

Protocol 8. Cat, female, 3.16 kgm.

- 9.49 a.m. 100 mgm. x kgm. apothetin in 10 per cent solution subcutaneously.
- 9.58 a.m. Moderate motor excitation.
- 9.59 a.m. Violent general clonic convulsion lasting more than one minute; not repeated.
- 10.02 a.m. Animal able to walk but still shows some motor excitation like that seen from morphine.
- 12.00 m. Seems quite normal.

Protocol 9. Cat, female, 2.28 kgm.

One hundred and twenty-five milligrams x kgm. tropacocain in 10 per cent solution subcutaneously. No symptoms developed.

Each of the drugs was tried in this way and five, or more than five, times the minimal fatal vein dose was survived when injected subcutaneously in the case of alypin, apothetin, beta-eucain,

nirvanin, procain, stovain, and tropacocain. Three and one-third times the fatal vein dose of cocain was survived after subcutaneous injection, while four times the fatal vein dose led promptly to death. Holocain showed a similar high toxicity by subcutaneous injection. It will be recalled that in an earlier section we stated that both holocain and cocain were less rapidly destroyed after intravenous injection than were the other members of the group. Both of these, however, are absorbed rapidly from the subcutaneous tissues so that the rate of absorption may exceed that of destruction with the result that the animal is fatally poisoned by a relatively small dose injected subcutaneously. Absorption of all the other members of the group, though rapid, does not keep pace with the capacity for destruction so that five or more times the fatal vein dose can be administered subcutaneously at one injection without causing death.

With the exceptions of one dose of 150 mgm. x kgm. of stovain and one of 50 mgm. x kgm. of cocain, the time between the injection of the drug and the appearance of the first symptom—where any effects at all were produced—averaged just over twelve minutes, with a minimum of seven and a maximum of sixteen minutes. After the dose of stovain cited as an exception the interval was twenty-five minutes, while after that of cocain it was twenty-eight minutes. A dose of 60 mgm. x kgm. of cocain, however, was followed in eight minutes by the first symptoms of its systemic action. When the doses given produced convulsions as part of their toxic actions the average interval between the administration of the dose and their onset was a little more than twenty-two minutes, with a minimum of nine and a maximum of eighty minutes. The eighty minute interval occurred following the dose of stovain just cited as requiring twenty-five minutes before the appearance of the first symptom and is the only one greater than twenty-five minutes. If it be omitted from the calculation of the average time to convulsions, the latter falls to fourteen minutes. One instance has not been included in either of the preceding computations, namely, that of a dose of 50 mgm. x kgm. of cocain. In this case no convulsions were observed in five hours, but more than

twenty-seven hours later the animal had one mild clonic convulsion. This experiment is so strikingly at variance with all of the others that we append the protocol.

Protocol 10. Cat, female, 2.6 kgm.

February 14, 10.26 a.m.	50 mgm. x kgm. cocain in 5 per cent solution subcutaneously.
10.54 a.m.	Emesis, repeated.
2.44 p.m.	Emesis.
5.00 p.m.	Animal has been depressed all afternoon.
February 15, 9.00 a.m.	Still somewhat depressed, refuses food.
2.00 p.m.	Mild clonic convulsion; not repeated.
February 16, 9.00 a.m.	Apparently normal.

In this experiment absorption began fairly promptly for emesis appeared twenty-eight minutes after the injection, and continued more slowly, for emesis was repeated at intervals for the next four hours. It is quite evident, however, that, slow as was the absorption, "essential" elimination was not able to keep pace with it for a clonic convulsion appeared twenty-seven hours after the injection, and we have not seen convulsions develop after intravenous injection of less than about two thirds of the fatal dose. In this instance "essential" elimination was much slower than one fatal vein dose per hour, as stated to be the usual approximate rate after intravenous injection, and illustrates the difference between cocain and the other members of the group.

Reference to the review of the literature of intoxication in man shows that the symptoms are much the same as those here recorded for cats; that they do not differ materially in nature for the several drugs; that the interval between the injection of the drug and the onset of symptoms is generally very brief in man, as in animals; and, finally, that recovery in man is quite rapid with all of the drugs except that after cocain it may be decidedly prolonged, just as in the case of cats. Thus after all of the drugs used, except cocain, recovery in man was almost invariably complete in an average of a little over two hours, the limits being half an hour and three hours and forty minutes.

No attempt has been made to fix the limits of the several time intervals for cats with great accuracy, gross differences between the several members of the group being alone of importance. Therefore the figures stated are not to be taken as exact indices of either the rapidity of absorption or of duration of action. Such a determination would have required far larger amounts of most of the drugs than we had at our disposal, and would have added little, if anything, of importance to the results.

We did, however, pursue this phase of the investigation further by observing the effects of giving repeated subcutaneous doses of several of the drugs at intervals of fifteen minutes. The results are shown in the accompanying protocols.

Protocol 11. Cat, male, 2.16 kgm.

- 10.04 a.m. 5 mgm. x kgm. holocain in 2 per cent solution subcutaneously; dose repeated at 10.19 and 10.34 a.m.
- 10.39 a.m. Emesis, depression, crying, tremulous.
- 10.48 a.m. Mild general convulsion, not repeated.
- 11.30 a.m. Seems fairly normal, sits up.
- 3.00 p.m. Eats ravenously, is entirely normal.

Protocol 12. Cat, female, 2.60 kgm.

- 10.06 a.m. 10 mgm. x kgm. cocain in 10 per cent solution subcutaneously; dose repeated at 10.21 and 10.36 a.m.
- 10.41 a.m. Nausea, slightly tremulous, pupils dilated.
- 10.51 a.m. 10 mgm. x kgm. as before.
- 11.02 a.m. Very hyperexcitable, almost in convulsions.
- 11.21 a.m. 10 mgm. x kgm. as before, no convulsions having developed.
- 11.30 a.m. Mild general clonic spasm, followed by tetanic spasm.
- 11.56 a.m. Spasms repeated up to now; animal now sitting up, head shakes continuously.
- 2.50 p.m. Emesis; is depressed.
- 4.00 p.m. Still depressed, refuses food, no other symptoms.
Normal following morning.

Protocol 13. Cat, female, 3.06 kgm.

- 10.00 a.m. 20 mgm. x kgm. apothetin in 10 per cent solution subcutaneously. Dose repeated at 10.15, 10.30, 10.45,

- 11.00, 11.30, and 11.45 a.m. and at 12.00 noon. Slightly tremulous and showed slight motor excitation after the fifth and seventh doses.
- 12.15 p.m. 30 mgm. x kgm. as before.
- 12.21 p.m. Slightly tremulous, otherwise normal. No further symptoms

These protocols illustrate the differences already mentioned between the relative rates of absorption and destruction of holocain and cocain as compared with other of the local anesthetics, including nirvanin, procain and stovain, protocols of which have been omitted. The subcutaneous administration of only one and one half times the fatal vein dose of holocain in three equal portions during thirty minutes was followed by a convulsion, and recovery seemed about complete an hour after the last dose. Three and one-third times the fatal vein dose of cocain, in five equal doses in one hour and fifteen minutes, produced serious intoxication and recovery was not complete after a lapse of four hours following the last dose.

A further, and striking, illustration of the slow "essential" elimination of cocain is to be found in Grode's paper. He tried to habituate various animals to cocain by repeated subcutaneous injection and he noted, in both cats and dogs, what he called an increased susceptibility to the later repetitions of a fixed dose, when these were made at intervals of one or two days. Thus, in one cat to which he attempted to give a daily subcutaneous dose of 20 mgm. x kgm. of cocain, the first dose caused salivation only; the second caused emesis; the third, three tetanic spasms; the fifth, eight tetanic spasms; then a two day interval intervened between the sixth and seventh doses, but the seventh dose caused repeated spasms and the eighth, on the following day, proved fatal in fifteen minutes.

In this experiment of Grode's it is to be noted that the cat was unable to destroy or eliminate as much as one and one-third times the fatal vein dose in the course of twenty-four hours when injected subcutaneously. This is a much slower rate of "essential" elimination than was indicated by our experiments

in which cocain was injected intravenously, and the difference is not readily explained.

In contrast with these we have administered about seven times the fatal vein dose of nirvanin in one and three-quarter hours, nine times the fatal vein dose of procain in two hours, and nine and one half times the fatal vein dose of apothessin in two and a quarter hours, without the production of convulsions in any instance. In all of these experiments, even after such very large doses, recovery was essentially complete within three hours and a half.

6. Influence of epinephrin on toxicity by subcutaneous injection

The results of the experiments discussed in the preceding section made us feel quite certain that the simultaneous injection of epinephrin and any of the local anesthetics would delay the onset of symptoms and diminish the toxicity of a given dose of the anesthetic by virtue of delayed absorption.

The results of all these experiments may be briefly summarized by saying that no symptoms followed doses of 100 and 150 mgm. x kgm. of nirvanin, 100 mgm. x kgm. of apothessin, or 50 mgm. x kgm. of alypin when injected subcutaneously in solutions containing 0.05 mgm. x kgm. of epinephrin in 1-10,000 dilution. A dose of 150 mgm. x kgm. of stovain with epinephrin caused nausea and emesis only, and they did not appear until one hour after the injection. A dose of 50 mgm. x kgm. of holocain with epinephrin was followed in thirteen minutes by the onset of violent clonic convulsions which were repeated many times during about five hours. The animal, however, recovered completely during the night and was apparently normal on the following morning. We have previously shown that the simultaneous administration of epinephrin greatly reduces the toxicity of procain by subcutaneous injection. In one experiment with alypin, the dose duplicating that previously mentioned, emesis followed thirty-seven minutes after the injection and clonic convulsions appeared two minutes later and were repeated for nearly an hour and a half. It is not clear why the epinephrin

failed to have a greater effect in delaying the absorption in this case, unless it be that this animal was more susceptible than usual to the irritant actions of the alypin. Finally, a dose of 85 mgm. x kgm. of cocain, with epinephrin, produced no symptoms in more than three hours, but the following day—eighteen and a half hours later—the animal was found in clonic convulsions which continued for four hours, up to the death of the cat.

These experiments also show that cocain and holocain are relatively slowly destroyed, so that even when absorption is delayed by the vasoconstrictor action of epinephrin they are still capable of accumulating in the essential organs to cause serious intoxication or death when five to six times the fatal vein dose is injected subcutaneously. On the other hand, the importance of the rapid destruction of the other members of the group is emphasized by these experiments with epinephrin, and it would seem to point to the decided value of always using epinephrin, as a routine, in combination with the cocain substitutes for subcutaneous injection in man to delay absorption and diminish the chances of accidental poisoning.

7. Notes on absorption from mucous membranes

Some experiments were performed to determine whether or not the local anesthetics are rapidly absorbed from the mucous membranes, especially those of the nose, urethra and bladder. Cocain and procain were employed chiefly, though a few experiments were made with apothecin. At first we attempted to apply a measured amount of a solution of procain to the cat's nasal mucous membrane by the insertion of a series of cotton plugs saturated with the solution. The small size of the nasal spaces, however, showed this method to be unsatisfactory and we therefore resorted to plugging the nares as far back as possible with small pledgets of cotton saturated with the solution to be tested. Then, with the cat restrained in a box and held with the head upward, small drops of the solution were instilled at frequent intervals into each nasal cavity until the desired amount had been introduced, or until symptoms developed.

The one objection to this method is that it does not entirely prevent some of the solution from running back into the pharynx and being swallowed. The following is a typical protocol.

Protocol 14. Cat, female, 2.8 kgm.

- 9.32-9.39 a.m. 53 mgm. x kgm. cocain in 10 per cent solution instilled into nose.
9.49 a.m. Tremulous, pupils dilated.
9.51 a.m. Clonic convulsion; repeated several times.
9.58 a.m. No further convulsions.
10.20 a.m. No evident symptoms.
11.05 a.m. Emesis.
1.00 p.m. Apparently normal.

Repeated clonic convulsions followed immediately after the instillation of 212 mgm. x kgm. of procain in 20 per cent solution into the nose of another cat, but the animal had recovered completely in two and a half hours. A third cat showed no symptoms after the instillation of 133 mgm. x kgm. of procain, partly into the nares and partly into the conjunctival sacs. In a fourth experiment 112 mgm. x kgm. of apothesis in 10 per cent solution were instilled into the nose, and the cat vomited once about half an hour later, but showed no other symptoms. While absorption was sufficient to permit of the development of some evidences of intoxication in three of the four experiments, it must be borne in mind that the doses employed were massive; thus the dose of cocain corresponds to 53 grains, or 3.5 grams, for a man of average size; that of procain which produced convulsions, to 212 grains, or nearly 14 grams; and that of apothesis, to 112 grains, or about 7 grams, for man. It is not surprising that some symptoms should have resulted from such doses, but rather that the intoxications produced were so mild as recorded.

Several instances of acute poisoning in man have been reported following the instillation of each of a number of the local anesthetics into the male urethra, and so frequent have been such cases following the use of cocain that special warning is given by many writers as to the dangers of thus anesthetizing

the urethra. This would seem to indicate rapid absorption from the urethral mucosa, but it is noteworthy that among the six cases of poisoning in man after intraurethral instillation of beta-eucain or alypin, which we have cited from the literature, the urethral mucous membrane had been damaged in four. Such a condition would naturally be expected to hasten the absorption, especially in such a highly vascular structure, and when the solution is introduced under considerable pressure, as is the case in most of such instillations. We undertook to study the absorption of some of these drugs from the urethra of the male cat, but found its capacity too small, as shown by the protocol of a control experiment.

Protocol 15. Cat, male, 2.16 kgm. (control)

Lightly anesthetized with ether, bladder exposed, blunt needle tied into anterior urethra and a concentrated solution of methylene blue instilled slowly. When 0.1 cc. of the solution had been injected it was seen to appear in the bladder, showing the small capacity of the cat's urethra.

It was evident from this experiment that large doses of the local anesthetics could not be kept within the cat's urethra, even when very concentrated solutions were employed, and that the injection was chiefly into the bladder. Nevertheless, the solution was made to pass through the urethra, and some of it did remain there, so we injected several cats in this way with large doses of the anesthetics. The results were invariably negative, that is, no absorption was evident in any of the seven experiments in which amounts of procain up to 150 mgm. x kgm.; of cocain, up to 90 mgm. x kgm.; and of apothessin, up to 100 mgm. x kgm. were thus instilled through the urethra into the previously emptied bladder. The entirely negative results of these experiments led us to abandon their further repetition.

We performed one experiment to discover whether the cat would absorb appreciable amounts of procain from the vagina. A blunt needle was inserted into the vagina of an unanesthetized cat and the orifice was plugged with cotton saturated with petro-

latum. Five hundred milligrams, or 277 mgm. x kgm., of procain in 50 per cent solution were instilled. No symptoms resulted.

These experiments on absorption from the mucous membranes indicate: That the local anesthetics are not more rapidly absorbed from the naso-pharyngeal mucous membranes of the cat than from the subcutaneous tissues. That in the cat the bladder, urethra and vagina absorb the local anesthetics very poorly.

8. *Elimination by the liver*

Wiechowski (19) sought to determine the fate of cocain in the animal organism by administering large doses orally to dogs and rabbits and examining the urine for cocain and ecgonin after collection for forty-eight hours. He found that dogs excreted an average of 5.1 per cent of the dose administered, the amounts recovered in the urine varying between none and 12.3 per cent. Rabbits, on the other hand, excreted none at all in the urine. He added cocain to the hashed livers or muscles, freshly excised, and agitated the mixtures for periods of four hours at the end of which he recovered about 80 per cent of the cocain originally added. From these experiments he concluded that the destruction of cocain should be regarded as a vital phenomenon. He also extracted the liver of a dog, weighing 5.8 kgm., which died half an hour after the administration of a total of 0.3744 gram of pure cocain. One hundred and eighty-three grams of the liver yielded 8 mgm. of cocain, an amount which corresponded to that which would have been present after uniform distribution of the drug throughout the body. He further determined that ecgonin does not appear in the urine of the dog or rabbit in appreciable amounts as a decomposition product of cocain.

Rifätwachdani (20) obtained quite different results for the excretion of cocain in the urine of rabbits after subcutaneous injection. He found that rabbits excreted in the urine in twenty-four hours amounts varying from 42 to 85 per cent of a single subcutaneous dose of 50 mgm. of cocain hydrochlorid, showing absence of marked destruction. The excretion of cocain was

studied in one rabbit receiving a daily subcutaneous dose of 50 mgm. of cocain. The amounts recovered in the urine on successive days rose from 33.6 to 112.1 per cent of the daily dose. In the whole experiment 65.8 per cent of the cocain administered was thus excreted. No ecgonin was found in the urine. The author also cites and confirms Kohlhardt's observation that long contact of cocain with the tissues, as by injection into a leg the circulation of which had been interfered with by constriction with a tourniquet, leads to a great reduction in its toxicity when the constriction is released. He tried to extract the cocain from such previously constricted legs, amputated two or three hours after the injection, and recovered from 20 to 100 per cent of that injected. He holds that this shows conclusively that there is no destruction or detoxification of cocain by the tissues, and suggests that the reduction in toxicity following injection into the constricted leg may be due to slow absorption and entrance into the general circulation, as is known to be the case with strychnin.

We showed in a previous paper that procain is rapidly destroyed during its perfusion through the cat's liver, and the same was shown to be true of stovain by Smith and Hatcher. In these two papers it was also shown that neither procain nor stovain is excreted in the urine of the cat in appreciable amounts. The results of the perfusion experiments were so clear cut and definite in demonstrating the destruction or fixation of these two local anesthetics by the liver that we decided to study the fate of several other members of the group in the same way. Two abbreviated protocols illustrate the methods of procedure and the results obtained.

Protocol 16. Cat, female, 3.44 kgm.

Under light ether anesthesia the animal was bled from the carotid artery while normal saline solution was being injected into a femoral vein. After defibrination the diluted blood measured 200 cc. In this we dissolved 600 mgm. of cocain hydrochlorid and divided the whole into a portion measuring 20 cc., which was reserved for control tests, and one measuring 180 cc., which was used for perfusing the liver.

The liver of the cat was removed at once after exsanguination, a cannula was tied into the portal vein and all the remaining blood was washed out with fresh, warm normal saline.

The liver was then perfused with the 180 cc. of diluted defibrinated blood, containing 540 mgm. of cocain, for one hour at a temperature of 37°C. The perfusion fluid passed through the liver more than 35 times, being oxygenated frequently in the intervals.

After perfusion 180 cc. of fluid were recovered, some dilution of the original having taken place with the saline which remained in the liver after washing.

Both the perfused fluid and the sample of defibrinated, diluted blood which was reserved for control were tested on cats by intravenous injection.

Tests

Control blood: Cat, male, 2.58 kgm.

11.20 a.m. 6 cc. x kgm. of the control blood were injected rapidly into femoral vein. Immediate respiratory stimulation, violent clonic convulsions, repeated every few minutes until 11.40 a.m.

11.50 a.m. Killed with chloroform.

Perfused fluid: Cat, male, 2.12 kgm.

11.27-11.28 a.m. 7.78 cc. x kgm. perfused fluid injected into femoral vein. Respiratory stimulation, one clonic convulsion.

11.29-11.31 a.m. 6.83 cc. x kgm. additional injected. One clonic convulsion.

11.33-11.34 a.m. 4.69 cc. x kgm. additional injected. Violent clonic convulsions began and were repeated just as in the control test.

Calculation

6.0 cc. of control blood contained 15 mgm. cocain.

19.3 cc. of perfused fluid, equivalent to 6.0 cc. of control, contains 15 mgm. of cocain.

180.0 cc. of perfused fluid contains 138.6 mgm. cocain.

Since 540 mgm. of cocain were originally present in the 180 cc., 401 mgm. were destroyed in the liver during perfusion.

Since the original perfusion fluid was somewhat diluted during passage through the liver, this figure is a little too high, but the result of

the experiment is to show conclusively that the liver destroyed or fixed not less than half of a total of 540 mgm. of cocain during perfusion for one hour, or an amount corresponding to about 5 fatal intravenous dose as for the cat from which the liver was removed.

Protocol 17. Cat, male, 3.56 kgm.

Bled and liver prepared as in preceding, and 1 gram of tropacocain, dissolved in a little normal saline, was added to the defibrinated blood and the whole made up to 250 cc. Portion of 50 cc. reserved for control tests; 200 cc. used to perfuse the liver.

The perfusion was carried on for half an hour, the fluid flowing through the liver as fast as possible. At least 40 complete circuits were made.

A test of the control fluid showed no destruction, while one of the perfused fluid indicated practically total destruction of the entire 800 mgm. of tropacocain originally present, or an amount equivalent to more than 10 times the single fatal vein dose for the animal from which the liver was removed.

The unused portion of the control sample of defibrinated blood was extracted and the extract tested on a cat. The test indicated recovery of practically all of the tropacocain originally present.

The liver, after perfusion, was then ground and extracted by the same method as that employed for the blood and tested on a cat. The test of the extract corresponding to one-third of the entire liver failed to reveal the presence of any tropacocain.

Perfusions similar to these were carried out with apothecin and nirvanin and showed that practically all of a total of 500 mgm. of apothecin and about two-thirds of a total of 900 mgm. of nirvanin were destroyed⁴ by the liver during perfusion for one hour. In all six of the nine different local anesthetics have been tested by perfusion through the excised liver and in every case it has been demonstrated that that organ is capable of destroying large amounts of these drugs rapidly. It is scarcely necessary to

⁴ We do not know with absolute certainty that the local anesthetics are actually destroyed in the liver, in the sense of chemical decomposition. There is a possibility that they may merely be fixed in an insoluble, or inert, form. All of the evidence, however, points to actual destruction by decomposition and we therefore prefer to speak of these drugs as being destroyed.

remark that we made control experiments to exclude destruction or other form of detoxification of the drugs by defibrinated, diluted blood, kept at body temperature and oxygenated during periods of time similar to those elapsing in the perfusions.

This rapid destruction of the local anesthetics by the liver accounts not only for the rapid recovery of the intact animal from the intravenous injection of doses which are just sublethal, but also for the fact that with most of the members of the group such doses can be repeated indefinitely at intervals of about twenty minutes until many times the single fatal dose has been injected. This destruction also explains the fact that when just sublethal doses are injected intravenously, even though respiration may have been stopped for one or two minutes, the animal will recover if the heart continues to beat and circulate the blood through the liver. The rapid destruction of these drugs also serves to explain in part the success of some of the resuscitative measures to be described later, as well as the ultimate recovery from more than average lethal doses after the previous administration of a cardiac stimulant drug.

It seems certain that man is also capable of rapidly destroying the anesthetics in his liver and that this largely accounts for his rapid recovery from severely toxic symptoms. Such destruction is a matter of prime importance in connection with the employment of antagonistic and resuscitative measures in cases of acute intoxication in man, for it is evident that if the vital functions of respiration and circulation can be maintained for even a short time there is always the chance of complete recovery through destruction of a portion of the drug which has been absorbed.

9. Efforts to influence the toxicity of the local anesthetics

One of the principal objects of our investigation of the local anesthetics having been the endeavor to throw some light upon the factors which favor acute intoxication in man and the methods of avoiding or overcoming accidental poisoning, we systematically investigated a number of agents and measures

which might be anticipated to exert some influence on the toxicity of these drugs. In many instances one or two experiments were sufficient to convince us that a lead was not worth following, so that we abandoned further work at once. In others we felt that we should make our experiments somewhat more extensive and searching, even though the results at first were negative.

(a) *Influence of atropin.* We performed a series of thirteen experiments to determine whether or not the intravenous injection of atropin just before that of one of the anesthetics would influence the toxicity of the latter through paralysis of the vagus endings in the heart or stimulation of the respiratory center. The dose of atropin employed was either 2 or 5 mgm. x kgm. and the interval between its injection and that of the local anesthetic varied from three to ten minutes. Cocain, stovain, procain and apothessin were tried as representatives of the group, doses both larger and smaller than those stated as the average fatal were administered. There was no evidence of any influence of atropin upon the symptoms produced by the local anesthetics, or upon the size of the fatal vein dose.

(b) *Influence of sodium bicarbonate.* Gros (21) showed that the addition of sodium bicarbonate to the commonly used salts of the anesthetic bases enhanced their anesthetic activity, due, he believed, to the better penetration of the nerves by the liberated bases than by their salts. Sollmann (22) summarizes a series of his own investigations and generally confirms Gros so far as the increase of anesthetic activity by the addition of sodium bicarbonate is concerned, but states that it is not certain whether such addition modifies their toxicity or not. We therefore investigated the influence of sodium bicarbonate on the toxicity of the various drugs of the group.

In all of the experiments the mixture of the local anesthetic with the sodium bicarbonate was made in the syringe just before injection, or in a graduate, and the solution, which usually showed some cloudiness or a fine precipitate, was vigorously shaken and drawn into the syringe for immediate injection. In every instance the dose of the bicarbonate was 25 mgm. x

kgm. in the form of a 5 per cent solution, while the local anesthetic was used in the customary concentration. All injections were made intravenously at the usual rate. The doses of the local anesthetics were usually 75 per cent of the average fatal, though in some cases the fatal doses were used, and once or twice doses larger than the average fatal were employed to determine whether there was a reduction in toxicity. In a few experiments repeated large doses of the anesthetic were given along with the alkali. The results, including the number of experiments performed, are shown in table 2.

TABLE 2

Showing results of simultaneous intravenous injection of local anesthetics with 25 mgm. x kgm. of sodium bicarbonate in cats

ANESTHETIC	NUMBER OF EXPERIMENTS	DOSES OF ANESTHETIC PER CENT OF FATAL	EFFECT ON TOXICITY
Procaïn.....	6	60-120	None
Nirvanin.....	3	80-100	None
Cocain.....	3	80-100	None
Apothesin.....	2	75	No increase
Stovain, beta-eucain, alypin, tropacocain.....	1 each	75	No increase

(c) *Influence of caffeine.* Caffein has been employed clinically in the treatment of acute cocain poisoning and Bastedo (23) cites Lieb as having found caffein effective in removing cocain heart block in the isolated turtle heart. It seemed possible, therefore, that it might prove somewhat antagonistic to the toxic actions of the local anesthetics in cats. Doses of 10 mgm. x kgm. of caffein were slowly injected intravenously and three minutes later one and a quarter times the minimal fatal dose of the local anesthetic to be tested was injected in the usual manner. Cocain, procain and apothésin were tried, but in no instance was there any evidence of antagonism, even when artificial respiration was employed to give the heart a chance to recover, nor was there alteration in the typical response to the local anesthetic. In one experiment we injected 3 mgm. x kgm. of caffein and 50 mgm. x kgm. of procain in the same solution with the

usual prompt onset of symptoms, followed by death in the typical manner.

(d) *Influence of hydrated chloral.* We noted in our previous paper two instances in which cats died promptly after the intravenous injection of as little as 9 and 10 mgm. x kgm., respectively, of procain, given during deep chloral narcosis. This suggested that hydrated chloral increases the toxicity of the local anesthetics, hence we decided to investigate the matter further. A series of nineteen experiments were performed and the results are shown in table 3.

These experiments indicate that severe poisoning by hydrated chloral may increase the susceptibility of the cat to the toxic actions of the local anesthetics, occasionally to a marked degree. On the other hand, degrees of poisoning by chloral short of the most severe do not as a rule seem to have much influence on the susceptibility to the local anesthetics. That the previous administration of hydrated chloral does exert some influence, both upon the immediate response to the local anesthetics, and upon

TABLE 3

Showing influence of hydrated chloral on toxicity of the local anesthetics for the cat

ANESTHETIC	DOSE		RATE OF INJECTION	NARCOSIS*	RESULT
	Mgm. x kgm.	Per cent of fatal			
Procain	9.0	21	5 minutes	Deep	Death
	10.0	24	Rapid	Deep	Death
	30.0	71	Rapid	Moderate	Recovery
	48.9	119	28 minutes	Deep	Recovery
	106.0	252	128 minutes	Deep	Recovery
Cocain	10.0	66	Rapid	Deep	Recovery
	10.0	66	Rapid	Deep	Recovery
	12.5	83	Rapid	Deep	Death
Tropacocain	15.0	75	Rapid	Mild	Recovery
	15.0	75	Rapid	Mild	Death
	15.0	75	Rapid	Mild	Death
Stovain	25.0	99	Rapid	Moderate	Death
	27.0	100	Rapid	Moderate	Death

TABLE 3—Continued

The following cats received repeated doses, all injections rapid

CAT	ANESTHETIC	DOSE		INTERVAL IN MINUTES	NARCOSIS	RESULT
		Mgm. x kgm.	Per cent of fatal			
A	Apothesin....	10.0	50	20	Mild	Recovery
		10.0	50			Recovery
		5.0	25			Recovery
B	Apothesin....	10.0	50	15	Mild	Recovery†
		10.0	50			Recovery
		15.0	75			Recovery
C	Apothesin....	15.0	75	20	Mild	Recovery
		15.0	75			Recovery
		15.0	75			Recovery
D	Stovain Procain....	20.0	72	20	Deep	Recovery
		35.0	83			Death
E	Stovain..... Apothesin..	20.0	72	22	Moderate	Recovery
		15.0	75			Recovery
F	Apothesin Procain....	15.0	75	30	Moderate	Recovery
		35.0	83			Recovery

* Deep narcosis is of such a degree that the animal cannot be roused; moderate is such that the animal can be roused partially by very rough handling and repeated painful stimuli, but lapses into sleep at once when left; mild is such that the animal remains in deep sleep when undisturbed, but can be roused fairly readily by rough handling, returning to deep sleep only after an interval of a few minutes.

In this connection we wish to emphasize the degree of intoxication produced by the chloral, even when classed as mild. Its severity is indicated by the fact that of 30 cats to which chloral was administered to produce narcosis, five died in their cages before the time for use and four died as a result of the handling incident to the operation.

† This animal received chloral the day preceding the injection of the anesthetic and was used without a further dose of chloral because it remained in a state of moderate to mild narcosis.

the rate of recovery is, however, clearly shown by the results of two parallel experiments, illustrated by figures 4 and 5. The figures carry all the data except the times required for complete recovery in the chloralized cat, which were approximately ten minutes after each of the three doses, contrasting with periods

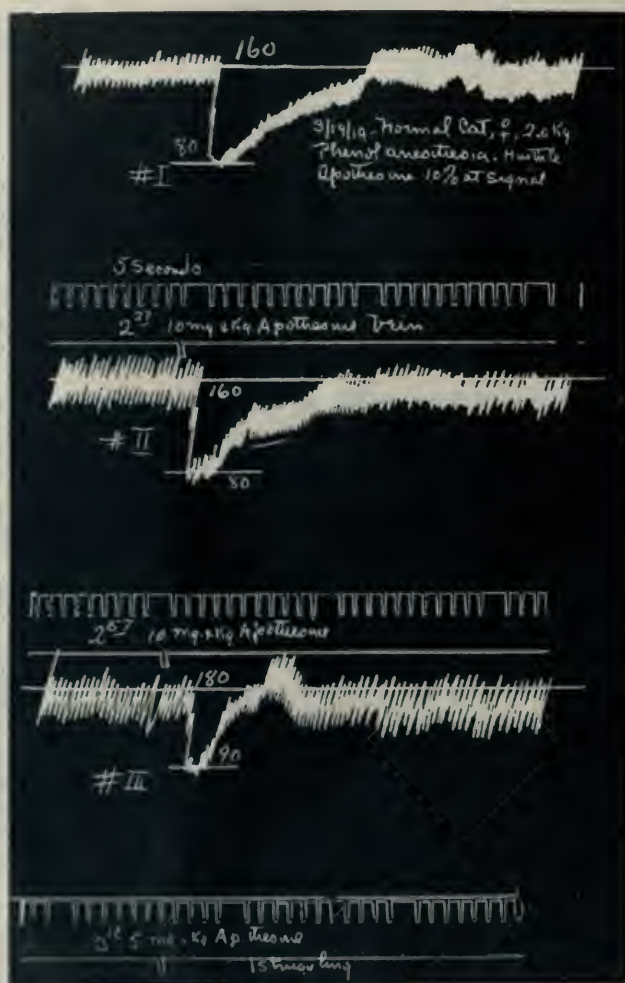


FIG. 4

of less than three minutes in the normal cat. The tracings show that after chloral the fall in blood pressure following the injection of the anesthetic is decidedly less abrupt than in the normal control, and while this effect was not always seen, it still was of frequent occurrence in those experiments in which we took graphic records. The slower recovery is almost certainly due to



Fig. 5

interference with the circulation whereby a longer time is required for destruction in the liver.

It is surprising that chloral did not show a more decided influence on the ultimate recovery from large doses of the local anesthetics, or upon the size of the fatal doses, in view of the material delay in the initial rate of recovery as shown in the blood pressure curves, and in view of the lower initial blood pressure in the chloralized than in the normal cat.

(e) *Influence of hemorrhage.* Attention has been called by several clinical writers to the fact that acute intoxication by the local anesthetics seems to be rather more likely to occur in ill-nourished, anemic persons than in the robust. A perusal of the literature, of accidental acute poisoning, however, does not show that the anemic and ill-nourished supply any more such occurrences than do others. Nevertheless, we investigated the influence of severe acute hemorrhage on the toxicity of several of these drugs by intravenous injection. The experimental method consisted in bleeding the cat from an artery to the extent of one-fourth of the total calculated blood volume. In from two to five minutes after the bleeding the anesthetic was injected into the femoral vein. The general results are shown in table 4.

Of the eleven cats receiving from 75 to 80 per cent of the fatal vein dose of the local anesthetics, seven died after typical symptoms and four recovered normally. Three cats receiving repeated injections, on the other hand, recovered from both doses in a manner indistinguishable from normal cats. It would seem, therefore, that severe acute hemorrhage may be regarded as diminishing the cat's ability to recover from large fractions of the average fatal vein dose of the local anesthetics in about half the instances. It is surprising that the effect of such a measure as the rapid withdrawal of one-fourth of the cat's total blood is not greater, for such a procedure not only materially reduces the blood pressure, but owing to the reduced blood volume tends to impair the function of all of the organs including the heart, the central nervous system and the liver. Such impairment might naturally be expected to both increase the cat's susceptibility to the local anesthetics and to reduce its capacity for destroying them in the liver.

TABLE 4

Showing the results of intravenous injection of local anesthetics in cats previously deprived of one-fourth of their total blood volume

ANESTHETIC	DOSE		RESULT
	Mgm. x kgm.	Per cent of fatal	
Cocain.....	12.5	83	Typical symptoms; death
	12.0	80	Typical symptoms; death
	12.0	80	Typical symptoms; recovery
	11.0	73	Typical symptoms; death
Apothesin.....	20.0	100	Typical symptoms; death
	15.0	75	Typical symptoms; recovery
Stovain.....	22.5	89	Typical symptoms; death
	20.0	72	Typical symptoms; recovery
Tropacocain.....	15.0	75	Typical symptoms; death
	15.0	75	Typical symptoms; death
	10.0	50	Typical symptoms; recovery

Each of the following cats received two doses

CAT	ANESTHETIC	DOSE		INTERVAL IN MINUTES	RESULT
		Mgm. x kgm.	Per cent of fatal		
A	Apothesin.....	10.0	50	20	Typical symptoms; recovery
		15.0	75		Typical symptoms; recovery
B	Apothesin.....	15.0	75	30	Typical symptoms; recovery
		15.0	75		Typical symptoms; recovery
C	Stovain.....	18.0	64	35	Typical symptoms; recovery
		22.0	88		Typical symptoms; recovery

(f) *Miscellaneous.* We planned to investigate the effects of nervous exhaustion in cats upon the toxicity of the anesthetics and sought to produce exhaustion by prolonged, intense excitement. We administered doses of 10, 10 and 20 mgm. x kgm. of procain at intervals of ten and fifteen minutes, respectively, to one such exhausted cat, but saw no definite evidence of an increased susceptibility to the drug. We then resorted to the

administration of camphor in doses sufficient to produce violent clonic convulsions which were repeated frequently during periods of two or more hours. When the convulsions were becoming infrequent and greatly diminished in severity owing to exhaustion we administered various doses of the local anesthetics, including procain, cocain, stovain and tropacocain, but observed neither increased susceptibility from the exhaustion, nor increased tolerance from the stimulant actions of the camphor.

We also sought to test the influence of surgical shock but after a single experiment we abandoned further efforts. In this experiment the cat was anesthetized lightly with ether and all of the measures commonly employed for producing shock were applied during a period of three-quarters of an hour, at the end of which time the cat's blood pressure had fallen only 28 per cent. The intravenous injection of 75 per cent of the fatal dose of apothecin resulted in death after four minutes, the respiration continuing feebly for nearly two minutes after the heart had stopped. No convulsions were observed following the dose of apothecin. While the result in this experiment is suggestive of an increased susceptibility due to the surgical shock, we did not feel justified in attempting its repetition owing to the difficulty in producing shock and to the extremely abnormal condition of the animal as a result of the manipulations.

Finally we sought to determine the influence of hypertonic saline in delaying the penetration of the local anesthetics into the cells and performed two experiments in which 25 cc. x kgm. of a 10 per cent solution of absolute sodium sulphate were injected intravenously just before the injection of procain, but there was no indication that this measure had any influence upon the toxicity of the anesthetic.

10. Synergism with epinephrin on systemic administration

It is well known that cocain and epinephrin have a synergistic action on the blood pressure, and we have called attention to the fact that procain is similarly synergistic with epinephrin, although, unlike cocain, it causes a fall in the blood pressure

when given alone. All of the other substitutes for cocain which we have investigated also cause a fall in blood pressure, and produce local vasodilatation when injected subcutaneously. We have tried all of the remaining local anesthetics with reference to their synergism with epinephrin and have found that it is present, though not always as marked as with cocain and procain. The synergism is often absent when the doses of the cocain substitutes are sufficient to cause even a slight initial fall of blood pressure, but when they are so small as to produce no appreciable effect, when given alone, the synergism with epinephrin is quite definite. It is not very marked with alypin and apothessin. The mechanism of this peculiar synergistic action is not understood and we hope to make it the subject of a more detailed investigation.

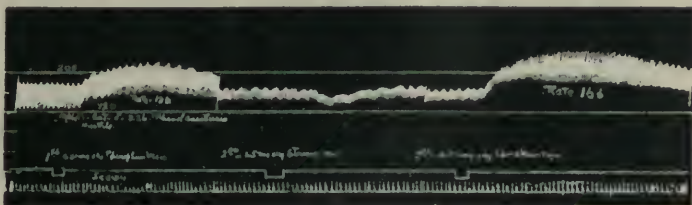


FIG. 6

Figure 6 illustrates this synergism, and it will be noted that the rise produced by a fixed dose of epinephrin is not only greater, but is also more sustained after the administration of the anesthetic than before, a condition precisely analogous to that shown in the case of procain.

11. Measures for resuscitation

(a.) *Artificial respiration, with or without cardiac massage.* In the search for methods of resuscitation we thought first of employing artificial respiration, or cardiac massage, or both together. It was apparent that if these measures should prove effective they should be capable of preventing death in the cat after the injection of appreciably more than the average fatal dose or they would be of relatively little value in cases of intoxi-

cation in man. We had two reasons for adopting this view; first, because in cases of accidental poisoning in man the amount of drug absorbed cannot be determined and it may often be well above the minimum which would be fatal in a given individual. Second, because the rapid destruction of the anesthetics in the liver should promptly reduce the amount of the drug free to act upon the heart and respiratory center to a point below that which would prove fatal, provided these two could be kept functioning for a few minutes.

Our method of procedure for determining the value of artificial respiration alone, or with cardiac massage, was to insert a tracheal cannula through a small incision after anesthetization of the skin with phenol, and to connect this with a respiration valve which was connected with an artificially interrupted air supply. We were thus ready to begin artificial respiration the moment that spontaneous breathing stopped, and could carry it on efficiently and uniformly for as long as might be desired. The drug to be used was injected rapidly into the femoral vein. When cardiac massage was employed it was given manually by rapid rhythmic compression and relaxation of the soft thorax over the heart, and proved to be adequate to maintain a fair blood pressure even after the heart had stopped (see tracings). We performed a series of eighteen experiments, the important points of which are shown in table 5. In every instance the attempts to resuscitate were continued until we were certain that the animal would not recover, generally for at least ten minutes.

The results of this series of experiments show that neither artificial respiration alone, nor combined with cardiac massage, is an efficient method for the resuscitation of cats after doses of the local anesthetics varying between 117 and 200 per cent of the fatal. In only two of the eighteen experiments did the cat recover, and in neither was cardiac massage employed, the heart resuming spontaneously after brief temporary cessation. It would seem probable that these two cats were naturally more tolerant to the local anesthetics than general, such tolerance having been noted occasionally.

TABLE 5

Showing results of applying artificial respiration and cardiac massage on recovery from large doses of local anesthetics

ANESTHETIC	DOSE		RESUSCITATIVE MEASURE	RESULT
	Mgm. x kgm.	Per cent of fatal		
Alypin.....	12.5	125	Artificial respiration	Death
Apothesin.....	25.0	125	Artificial respiration	Recovery
	40.0	200	Artificial respiration	Death*
Beta-eucain....	15.6	125	Artificial respiration	Death
	18.7	150	Artificial respiration; cardiac massage	Death
Cocain.....	20.0	133	Artificial respiration; cardiac massage	Death
Holocain.....	12.5	125	Artificial respiration	Death
	12.5	125	Artificial respiration; cardiac massage	Death
Nirvanin.....	37.5	117	Artificial respiration	Death
	37.5	117	Artificial respiration; cardiac massage	Death
Procain.....	60.0	142	Artificial respiration	Death
	60.0	142	Artificial respiration	Death
	60.0	142	Artificial respiration	Death
	60.0	142	Artificial respiration	Death
	60.0	142	Artificial respiration	Death
Stovain.....	37.5	135	Artificial respiration	Death
	37.5	135	Artificial respiration; cardiac massage	Death
Tropacocain....	25.0	125	Artificial respiration	Recovery

* This was the same animal which received the dose of 25 mgm. x kgm. of apothesin one hour earlier.

(b) *Influence of epinephrin plus artificial respiration and cardiac massage.* We stated in our earlier paper on procain that the simultaneous intravenous injection of epinephrin interfered to some extent with the toxic action of procain, and we there recorded one instance of a cat's surviving a dose of procain 50 per cent larger than that usually fatal when it was injected simul-

taneously with epinephrin. Those observations suggested the further study of the value of epinephrin as an aid to resuscitation, and we performed a series of seventeen experiments which are illustrated by the following representative protocol and accompanying tracing—figure 7.

*Protocol 18. Cat, male, 3.34 kgm.**

Skin anesthetized with phenol and connection made with tracheal cannula for artificial respiration. Cannula into left femoral vein for injection of epinephrin in 1-100,000 solution. Normal tracing.

10.30 a.m. 18.7 mgm. x kgm. beta-eucain into right femoral vein.

Prompt cessation of respiration and heart, blood pressure fell to zero. Artificial respiration and injection of epinephrin begun at once. Cardiac massage twice to carry epinephrin to heart. Prompt recovery of heart; artificial respiration stopped after about three minutes. Complete recovery.

11.30 a.m. 12.5 mgm. x kgm. beta-eucain as before. No attempt to resuscitate. Typical death, except for temporary partial recovery of heart.

The importance of carrying on artificial respiration in addition to the injection of epinephrin is illustrated by another experiment, the details of which are shown on the graphic record—figure 8. This experiment shows the beginning recovery of the heart about two and one half minutes after a dose of apothecin twice that usually fatal. The heart then improved rapidly and the blood pressure rose above the level previous to injection. Cessation of artificial respiration was followed by a rapid fall in the blood pressure with weakening of the heart. Reinstitution of artificial respiration rapidly restored the heart. This phenomenon was repeated once more, after which recovery was complete. Neither of the two temporary interruptions of artificial respiration was followed by the resumption of spontaneous breathing. This was probably the result of the direct action on the respiratory center of the very large dose of apothecin, and was the probable cause of the beginning failure of the heart on both occasions.



Fig. 7



Fig. 8

TABLE 6

Showing effects on toxicity of large doses of the local anesthetics from the intravenous injection of epinephrin combined with artificial respiration and cardiac massage

ANESTHETIC	DOSE		EPINE- PHRIN 1-100,000 cc. x kgm.	ARTIFICIAL RESPIRA- TION	CARDIAC MASSAGE	RESULT
	Mgm. x kgm.	Per cent of fatal				
Alypin.....	12.5	125	7.7	+	+	Recovery
Apothesin.....	25.0	125	7.5	+	0	Recovery
	30.0	150	9.3	+	+	Recovery
	40.0	200	13.0	+	+	Recovery
Beta-eucain.....	18.7	150	6.0	+	+	Recovery
Cocain.....	20.0	133	8.0	+	0	Recovery
	22.5	150	8.1	+	+	Death
Holocain.....	12.5	125	9.4	+	0	Death*
Nirvanin.....	37.5	117	5.5	+	+	Recovery
Procain.....	50.0	120	8.2	+	+	Death
	50.0	120	11.7	+	+	Recovery†
	60.0	142	8.0	+	+	Recovery
	80.0	190	9.0	+	+	Death‡
Stovain.....	37.5	135	6.1	+	+	Death
	37.5	135	2.5	+	+	Recovery
Tropacocain.....	25.0	125	9.9	+	+	Recovery
	30.0	150	2.2	+	+	Death

* Recovery was apparently complete and resuscitative measures were stopped. Heart and respiration failed together five minutes later.

† This cat had received the dose of apothesis of 30 mgm. x kgm., recorded above, half an hour previously.

‡ Recovery was apparently complete in ten minutes and all resuscitative measures were stopped fifteen minutes after injection of the anesthetic. Spontaneous respiration was not resumed and heart began to fail after about one minute, but was restored promptly by reinstitution of artificial respiration. This was continued for nine minutes when spontaneous breathing began. Nine minutes later the animal died, the respiration failing before the heart. The cat in this experiment had received the dose of 60 mgm. x kgm. of procain, recorded above, only twenty-two minutes earlier.

The chief points in the several experiments in this series are shown in table 6, from which it is evident that the combination of the intravenous injection of epinephrin with effective artificial respiration, with or without resort to cardiac massage, is usually capable of saving the cat from doses of the local anesthetics varying from 117 to 200 per cent of the average fatal. Death resulted in only six of the seventeen experiments, and in one of these it followed the administration of nearly twice the fatal dose of the anesthetic—procain—to a cat which had received almost one and a half times the usual fatal dose of the same drug only twenty-two minutes previously. Control experiments showed that normal saline was without value in antagonizing the local anesthetics.

While cardiac massage cannot be carried out so efficiently in man as in the cat, it can be performed sufficiently to cause the greater part of an intravenous injection of epinephrin to reach the heart; and several satisfactory methods are available for carrying on artificial respiration in man. These measures, also, are all such as can be applied without loss of time under most conditions and they certainly seem to be the most effective means at our disposal for saving life after the development of symptoms of acute intoxication by the anesthetics of this group. Since acute poisoning in man is accidental and occurs when least expected, those who frequently employ these local anesthetics should always be prepared to apply the three resuscitative measures of intravenous infusion of epinephrin, artificial respiration and cardiac massage, and all three should be used in combination since no one alone is effective except in rare instances.

12. Antagonism by ouabain

The fact that the several local anesthetics promptly bring the cat's heart to standstill has been shown to be characteristic of their acute toxic action, but this paralysis, while immediate, is not permanent in all cases, even after fatal doses, for we not infrequently observe a temporary, though rather feeble, spontaneous return of the heart beat, as illustrated in figure 3. These

facts, combined with the rapid destruction of the anesthetics by the liver, prompted us to try the effect upon their toxicity of the previous stimulation of the heart by one of the digitalis bodies. For the sake of convenience we selected ouabain, and also because it has a fairly well fixed fatal dose upon which we could base our calculations. We administered 60 per cent of the fatal dose of ouabain to several cats, either intravenously or intramuscularly, and after intervals of fifteen minutes to several hours, gave more than an average fatal dose of one of the local anesthetics in the usual manner. Artificial respiration was employed in most cases to tide over the period required for recovery of the heart, for we found that without it the cat would die from the respiratory paralysis even when the heart was evidently recovering. Our first four experiments were made with procain and we present an abbreviated protocol to illustrate the results.

Protocol 19. Cat, male, 2.94 kgm.

- 9.51 a.m. 0.06 mgm. x kgm. ouabain intravenously.
10.21 a.m. 60.00 mgm. x kgm. procain intravenously in 20 per cent solution. Prompt convulsion; respiration stopped in about one minute; heart feeble, but continued to beat. Artificial respiration started at once and continued for five minutes, during which the heart rapidly recovered. Complete recovery within fifteen minutes.

Although these first experiments were controlled by the administration of the same dose of procain to two normal cats with prompt death, in spite of artificial respiration, the results were so striking that we undertook a more rigid control. For this purpose one of us injected four cats with either normal saline, or with 0.06 mgm. x kgm. of ouabain, and the other then administered 60 mgm. x kgm. of procain to each, carrying out artificial respiration in all, and was able to identify correctly the two cats which had received ouabain by the fact that they recovered while the other two died promptly.

TABLE 7

Showing effect on toxicity of the local anesthetics of the previous administration of ouabain, supplemented by the use of artificial respiration

ANESTHETIC	DOSE		DOSE OF OUBAIN		ARTIFICIAL RESPIRA- TION	RESULT
	Mgm. x kgm.	Per cent of fatal	Mgm. x kgm.	Per cent of fatal		
Apothesin.....	30.0	150	0.01	10	+	Death
	30.0	150	0.02	20	+	Death
	30.0	150	0.06	60	0	Recovery*
	40.0	200			+	Death†
	35.0	175	0.06	60	+	Recovery
Beta-eucain.....	18.7	150	0.06	60	+	Recovery*
Cocain.....	20.0	133	0.06	60	+	Death†
	20.0	133	0.06	60	+	Recovery*
Holocain.....	12.5	125	0.06	60	0	Recovery*
Nirvanin.....	37.5	117	0.01	10	+	Death†
	37.5	117	0.02	20	0	Recovery*
	40.0	125	0.06	60	0	Recovery*
Procain.....	60.0	142	0.02	20	+	Recovery
	60.0	142	0.06	60	0	Death
	60.0	142	0.06	60	+	Recovery
	60.0	142	0.06	60	+	Recovery
	60.0	142	0.06	60	+	Recovery
	60.0	142	0.06	60	+	Recovery§
	60.0	142	0.06	60	+	Recovery§
Stovain.....	37.5	135	0.02	20	+	Recovery
	37.5	135	0.06	60	+	Recovery
	40.0	148	0.06	60	+	Death‡¶
Tropacocain.....	25.0	125	0.05	50	+	Recovery

* In the experiments thus marked the heart did not stop.

† This animal had received the preceding dose of 30 mgm. x kgm. of apothesis 55 minutes earlier.

‡ In the experiments thus marked cardiac massage was employed.

§ In these two experiments the cats were identified as having received ouabain, unknown to the experimenter—see text.

¶ This animal had been given the dose of 40 mgm. x kgm. of nirvanin, recorded above, one hour earlier.



FIG. 9



FIG. 10

Table 7 gives the important details of each of this series of twenty-three experiments and shows that the administration of a dose of 0.02 mgm. x kgm. or over of ouabain effectively protects the heart from permanent stoppage by doses of the local anesthetics ranging from 117 to 175 per cent of the average fatal. Of the twenty-one experiments in which 0.02 mgm. x kgm. or more of ouabain was given, death followed the administration of the local anesthetic in only five. Of these five deaths, one followed the administration of twice the fatal dose of apothecin to a cat which, fifty-five minutes before, had been given a dose of the same drug 50 per cent greater than the average fatal and had recovered. A second death followed the administration of 148 per cent of the fatal dose of stovain to a cat which had survived 125 per cent of the average fatal dose of nirvanin given an hour earlier. Excluding these two, death followed the administration of these large doses of the local anesthetics, given after the previous administration of 20 to 60 per cent of the fatal dose of ouabain, in only three out of nineteen cats. Artificial respiration was employed in all but five of the experiments, in four of which it was not necessary, while in one it was omitted purposely. Cardiac massage was tried in three of the experiments in which the heart did not show evidences of recovering, but it was without effect. By way of graphic illustration of this antagonism we introduce figures 9 and 10, taken from two parallel experiments.

DISCUSSION

One fact of fundamental importance has emerged from the investigations detailed in the preceding pages; namely, that the "essential" elimination of all of the local anesthetics studied, excepting cocain and holocain, proceeds with great rapidity and is completed within a few minutes following the intravenous injection of a sublethal dose. The "essential" elimination of cocain and holocain is a much slower process, and in the case of cocain it may not be complete after periods of one to two, or more, days. The elimination of all of the local anesthetics in the cat has been shown to be accomplished by their destruction

in the liver, as demonstrated by perfusion of that organ with solutions of the several drugs. In view of the close similarity of his behavior to toxic doses of the local anesthetics, it is highly probable that man also eliminates these drugs in the same way as the cat, especially since such rapid elimination by the kidney is most unlikely.

The local anesthetics can be divided into two groups according to their rates of destruction or "essential" elimination in the cat: Group 1, rapidly eliminated, includes alypin, apöthesin, beta-eucain, nirvanin, procain, stovain and tropacocain. Group 2, slowly eliminated, includes cocain and holocain. This grouping apparently applies also for man.

The prompt recovery of the cat following the intravenous injection of a just sublethal dose of any of the members of group 1 is due to the rapid destruction of those drugs, while slower destruction in the case of cocain and of holocain explains the less rapid and less complete recovery after corresponding doses.

The ability of the cat to withstand repeated intravenous injections of large fractions of the minimal fatal vein doses of members of group 1, when given at intervals of fifteen to twenty minutes, or to survive the slow and continuous injection of several times the average fatal vein dose, depends upon the rapid "essential" elimination of those drugs, and the inability to withstand similar injections of corresponding amounts of the drugs of group 2 is due to their slower elimination.

Rapid "essential" elimination of the members of group 1 accounts for their relatively low toxicity by subcutaneous injection, or after their application to mucous membranes, in the cat and other animals, in spite of the fact that they are absorbed rapidly from the subcutaneous tissues and from some mucous surfaces. It also explains the marked reduction in the toxicity of these drugs effected by the delayed absorption produced by the simultaneous injection of epinephrin. Slower "essential" elimination of the members of group 2 accounts for their relatively higher toxicity after subcutaneous injection, or application to mucous membranes, and for the lesser reduction in their toxicity effected by epinephrin.

Man also apparently recovers more rapidly from non-fatal acute poisoning by the members of group 1 than from cocain, the only member of group 2 on which we have data, and the difference is almost certainly again referable to his ability to destroy the drugs of the first group more rapidly and completely than he can cocain.

Finally, the successful resuscitation of cats, after intravenous doses of 117 to 200 per cent of the average fatal, by means of artificial respiration and stimulation of the heart by epinephrin, and the protection of cats from death, following doses of 117 to 175 per cent of the average fatal, by the previous stimulation of the heart by ouabain and the brief subsequent employment of artificial respiration, are both due primarily to the rapid destruction of the local anesthetics, by which the excess administered is promptly eliminated.

SUMMARY

1. The more important literature of acute intoxication in man from the local anesthetics is reviewed, and cases are cited to show the close similarity between the symptoms produced in man and in the lower animals, especially the cat.
2. The phenomena of acute intoxication in the cat, following the intravenous injection of the local anesthetics, are described, and the similarity of the phenomena produced by different members of the group is emphasized.
3. The maximum toxicity for each of the members of the series has been determined by rapid intravenous injection in the cat, and the relative toxicities of the several drugs are represented graphically.
4. The several different local anesthetics are shown to be mutually and quantitatively synergistic, so far as their fatal actions are concerned.
5. The capacity of the cat to withstand the intravenous injection of several times the fatal vein dose of any of the local anesthetics, except cocain and holocain, has been shown by repeated injections of large doses, or the continuous injection of relatively dilute solutions.

6. The toxicity of the local anesthetics for the cat, after subcutaneous injection, has been shown to depend upon the ratio between the rate of absorption and that of elimination, and the local anesthetics can be divided into two classes with reference to that ratio. Five, or more than five, times the minimal fatal vein dose of alypin, apothecin, beta-eucain, nirvanin, procain, stovain and tropacocain can be injected subcutaneously in the cat without causing death, while four, or less than four, times the fatal vein doses of cocain and holocain similarly injected prove fatal.

7. The simultaneous subcutaneous injection of epinephrin with the local anesthetics materially reduces the toxicity of the latter by delay in the rate of their absorption, but this reduction is much less marked in the cases of cocain and holocain than with the other members of the series, and is referable to their much slower "essential" elimination.

8. The absorption of several of the local anesthetics from the mucous membranes of the nose and pharynx of the cat has been shown to be no more rapid than from the subcutaneous tissues, and the urethra, bladder and vagina resist the absorption of these drugs to a great extent.

9. The elimination of the local anesthetics in the cat has been demonstrated to be due to their rapid destruction by the liver, and this takes place in the excised, perfused organ as well as in the liver of the intact animal.

10. Various efforts have been made to influence the toxicity of the local anesthetics for the cat, and severe acute hemorrhage and narcosis by hydrated chloral alone seem to have any material influence. Both of these measures tend to increase the cat's susceptibility to the toxic actions of the local anesthetics, probably by diminishing the rate of their destruction in the liver through impairment of the circulation.

11. All of the local anesthetics have been shown to be synergistic with epinephrin on the blood pressure in a manner analogous to cocain.

12. The employment of artificial respiration, combined with stimulation of the heart by the intravenous injection of epi-

nephren, is capable of saving cats from death following the intravenous injection of as much as twice the average fatal dose of the local anesthetics.

13. Stimulation of the heart by the previous injection of ouabain permits the cat to recover from intravenous injections of nearly twice the average fatal dose of the local anesthetics, when the temporary paralysis of the respiratory center is combated by the use of artificial respiration.

14. The success of the last two measures depends upon the rapid destruction of the local anesthetics by the liver.

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THE APPLICATION OF THE KJELDAHL METHOD TO COMPOUNDS OF BRUCINE, WITH REFERENCE TO THE BRUCINE SALT OF A NEW NUCLEOTIDE.

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The alkaloids have come into considerable prominence in physiological chemistry because they have been found to yield crystalline salts with various organic acids whose analysis might be expected to determine definitely the chemical composition of the acids in question. In one of the most recent articles on the subject Dakin states that he has prepared from his newly discovered amino acid (oxyglutamic acid) a series of beautifully crystalline salts with strychnine, brucine and other alkaloids, and that a further study of these products may prove of service.¹

Brucine has been an almost indispensable reagent in dealing with nucleotides. All substances of this class form difficultly soluble brucine salts whose recrystallization from various solvents leads to products of a purity that can be secured in no other way. Such a product was recently obtained in this laboratory. The nucleotide was prepared from yeast nucleic acid by partial oxidation with potassium permanganate. Upon neutralization of its hot aqueous solution with brucine in substance a brucine salt was formed which was deposited in characteristic needles as the solution cooled. This brucine salt is very characteristic, and entirely unlike any brucine salt that the writer has ever seen. It may be recrystallized from water or alcohol of various strengths as often as one desires without suffering the least alteration in its characteristic solubilities or crystalline form.

The nucleotide from which this brucine salt was prepared contains a purine group and a pyrimidine group, so that the

¹ H. D. Dakin, *Biochemical Journal*, xii, 1918, 290.

brucine salt should be one of two things: (1) The brucine salt of a mixed dinucleotide; (2) a mechanical mixture of the brucine salts of two mononucleotides, one a purine nucleotide and the other a pyrimidine nucleotide.

The method of deciding between these alternatives seems simple. The nucleotide should be prepared from the brucine salt, submitted to hydrolysis with sulphuric acid, and the ratio found between its easily split phosphoric acid and its firmly bound phosphoric acid. If this ratio is found to be fractional, then we are dealing with a mechanical mixture, but if the ratio is found to be unity, the two nucleotides are present in equivalent quantities and we are dealing with a dinucleotide. Unfortunately, the procedure is not experimentally possible in this particular case. After removing the brucine from the brucine salt with ammonia and the subsequent conversion of the ammonium salt (through the lead salt) into the free nucleotide, one obtains an aqueous solution which on concentration in a vacuum deposits beautiful needles of adenine nucleotide. At the same time there remains in the solution a second nucleotide which decomposes easily as the solution is further concentrated even in a vacuum at 40°. This decomposition produces free phosphoric acid but no purine compound, and the darkening of the solution indicates that pentose has been formed. From a number of considerations the provisional conclusion has been drawn that the crystalline brucine salt under discussion is the brucine salt of adenine-cytosine dinucleotide, and if this view of the case should finally prove correct then free compound nucleotides are much more easily decomposable into their constituent mononucleotides than is commonly supposed, although their brucine salts are comparatively stable. In dealing with compound nucleotides we are therefore forced to the analysis of their crystalline brucine salts, and it seems that the individuality of a compound nucleotide will be finally decided not so much by our ability or inability to separate it into its constituent mononucleotides but more by a knowledge that it does or does not contain its constituent mononucleotide groups in exactly equivalent quantities.

The necessity of proceeding in this manner has been apparent for a long time, but the analysis of brucine salts of nucleotides by methods sufficiently refined to give results of value has not been possible. In the first place the brucine salts of all nucleotides (whether mononucleotides or polynucleotides) have practically the same percentage of carbon, hydrogen and phosphorus. Again, free nucleotides differ quite markedly from one another in their nitrogen percentages, but this difference is to a large extent lost in their brucine salts because brucine itself contains nitrogen and has so high a molecular weight (394). It therefore seems necessary to find an exact method for nitrogen analysis which may be applied to this series of compounds. The Dumas method is laborious, has not nearly the exactness required, and cannot be used at present owing to the impossibility of securing the necessary reagents. For these reasons a modification of the exact Kjeldahl method was sought which could be used. A weighed portion of the brucine salt mentioned above was digested for five hours with 10 cc. of concentrated sulphuric acid, 7.5 grams of potassium sulphate, and a few drops of copper sulphate. The ammonia was distilled off and determined in the ordinary way. As this experiment gave a very rational result others were made in which the digestion was of shorter duration. All gave the same percentage of nitrogen which is that required for cytosine-adenine dinucleotide. The anhydrous substance was analysed.

- I. 0.5116 required 13.94 cc. of standard sulphuric acid (1 cc. = 0.03641 N). Digestion 5 hours.
- II. 0.5082 required 13.90 cc. of standard sulphuric acid. Digestion 4 hours.
- III. 0.5596 required 15.23 cc. of standard sulphuric acid. Digestion 3 hours.
- IV. 0.4981 required 13.53 cc. of standard sulphuric acid. Digestion 2 hours.
- V. 0.4878 required 13.34 cc. of standard sulphuric acid. Digestion 1 hour.

NITROGEN REQUIRED FOR ADENINE NUCLEOTIDE	NITROGEN REQUIRED FOR CYTOSINE NUCLEOTIDE	NITROGEN REQUIRED FOR ADENINE CYTOSINE DI-NUCLEOTIDE	NITROGEN FOUND
11.10	8.89	10.01	I. 9.92 II. 9.96 III. 9.91 IV. 9.89 V. 9.96

In order to check these results determinations of nitrogen in pure anhydrous brucine sulphate were made by the same method.

- I. 0.5221 required 9.06 cc. of standard acid. Digestion 5 hours.
- II. 0.5164 required 8.88 cc. of standard acid. Digestion 4 hours.
- III. 0.4972 required 8.55 cc. of standard acid. Digestion 3 hours.
- IV. 0.5330 required 9.19 cc. of standard acid. Digestion 2 hours.
- V. 0.5007 required 8.72 cc. of standard acid. Digestion 1 hour.

NITROGEN REQUIRED	NITROGEN FOUND
6.32	I. 6.32 II. 6.27 III. 6.26 IV. 6.28 V. 6.34

Some pure brucine sulphate was dissolved in hot water and the free alkaloid was precipitated in fine needle clusters by the careful addition of ammonia. The brucine was dehydrated at 105° and analysed.

- I. 0.4073 required 7.93 cc. of standard acid. Digestion 5 hours.
- II. 0.5572 required 10.86 cc. of standard acid. Digestion 4 hours.
- III. 0.5617 required 11.00 cc. of standard acid. Digestion 3 hours.
- IV. 0.5022 required 9.79 cc. of standard acid. Digestion 2 hours.
- V. 0.3527 required 6.85 cc. of standard acid. Digestion 1 hour.

NITROGEN REQUIRED	NITROGEN FOUND
7.11	I. 7.09 II. 7.10 III. 7.13 IV. 7.10 V. 7.07

The analytical data show that the Kjeldahl method gives exact results with salts of brucine.

IODIN: EFFECT ON FIBROUS NODULES

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From the Department of Pharmacology of the Medical School of Western Reserve University, Cleveland

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Active counter-irritation by means of iodine has long been used to hasten the absorption of persistent inflammatory swelling. It has been difficult, however, to subject their reputation to an experimental test. I have found a suitable test object in the fibrous nodules that follow intracutaneous injections in the wheal-method of testing local anesthetics (1). These nodules persist in the human skin, practically unchanged for three to four weeks, and then fade slowly, being practically gone in six to ten weeks, exceptionally, they may be seen for even longer periods.

The injections are usually of 0.2 to 0.3 cc. The solutions used in these injections are made practically isotonic with sodium chlorid. The nature and concentration of the anesthetics, as ordinarily used in the tests, has no effect on the appearance or duration of the nodules.

The following results show that the absorption of the nodules is materially hastened by the application of iodine, providing this is sufficiently energetic; but that no effect whatever is produced by mild iodine application.

On a given day, 27 wheals were produced on the left fore-arm, i.e., 9 rows of 3 wheals. On the same day, 3 rows of 3 wheals were made on the right arm.

The application of iodine to the left arm was started 6 days later; the nodules being prominent and firm and practically undistinguishable. Alternate rows were left untreated. To the others, a 7 per cent alcoholic solution of iodine was applied once daily as follows. The days are always reckoned from the day on which the anesthetics were injected.

Iodin row 1: One application on sixth day. This caused very little irritation.

Iodin row 2: Five applications from the sixth to the tenth day. The later applications caused considerable smarting, and were followed by peeling of the skin.

Iodin rows 3 and 4: Eight applications, from the sixth to the thirteenth day. The later applications were distinctly painful, especially in row 4.



FIG. 1. PHOTOGRAPH OF PLASTER CAST OF SKIN-NODULES AFTER IODIN TREATMENT

The numbered rows received iodine treatment of increasing duration. The unnumbered rows are untreated rows.

The results are seen from the photographs of a plaster-cast¹ taken on the twenty-third day (i.e., the eighteenth day after the first iodine treatment). This shows no effect from the one and five applications (iodine rows 1 and 2). Both of the eight-treatments show distinct effects. Iodine row 3 was slightly flatter

¹ I am indebted to Prof. T. W. Todd for the cast and photograph.

than its neighbors, although this is not well shown on the photograph. In iodine row 4 in which the iodine irritation had been most severe, the nodules had entirely disappeared.

At the present time, fifty days after the injection, these differences are still plainly visible, although the nodules have nearly disappeared.

The nodules of the right arm were used in an attempt to determine whether a single very energetic iodine treatment would be as effective as the cumulative results of the reported milder treatments. For this purpose, a row of nodules was given 10 heavy applications of 7 per cent alcoholic iodine on the twenty-seventh day. At this time all the nodules had begun to recede somewhat in size. The treatment caused rather severe pain and considerable swelling, the irritation being much greater than with the spaced applications of the left arm. The soreness persisted for two days, and the skin peeled. Notwithstanding this energetic treatment, the nodules were not materially affected.

CONCLUSIONS

1. Intradermal fibrous nodules, produced by intracutaneous injections, furnish practical control objects for testing the absorptive efficiency of local applications of iodine.
2. Very definite effects were obtained by daily applications, continued at least eight days, and pushed to considerable irritation of the skin.
3. Treatment over shorter periods was inefficient, even when the dosage and the irritation were greater.

REFERENCE

- (1) SOLLMANN, T. Anesthesia of the human skin. *J. Pharm. Exp. Ther.*, xi, 69, 1918.

SCIENTIFIC PROCEEDINGS OF THE AMERICAN SOCIETY FOR PHARMACOLOGY AND EX- PERIMENTAL THERAPEUTICS

TENTH ANNUAL MEETING HELD AT BALTIMORE, APRIL 24-26,
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Edited by the Secretary, Dr. E. D. Brown

Acetyl Salicylic Acid and Heat Regulation in Normal Individuals. H. G. BARBOUR AND N. M. DEVENIS. From the Department of Pharmacology, Yale University School of Medicine.

The effects of 1 gram of acetyl salicylic acid have been studied upon normal individuals during absolute muscular rest in a Benedict respiration chamber.¹ The drug was administered by mouth with 250 cc. of water six hours after a very light breakfast. The observations began after a preliminary period of one hour. The heat production was calculated from three or more successive half-hourly determinations of CO₂ excretion. Heat elimination was estimated from the changes in rectal temperature. (These changes afford an approximation of differences in average body temperature although the latter cannot be determined absolutely.) The following results have been obtained:

FIVE NORMAL SUBJECTS	CALORIES PER SQUARE METER PER HOUR				BODY TEMPER- ATURE CHANGE
	Produced		Eliminated		
	Control	A-S.A.	Control	A-S.A.	
Average of six experiments.	36.92		38.68		°C. -0.09
Average of five experiments		40.28		39.3	+0.03
Change induced by drug		+9.1%		+1.7%	

In both series of experiments the pulse rate fell at the rate of one beat per minute every half hour.

It is concluded that 1 gram of acetyl salicylic acid produces in most normal adults a definite increase in CO₂ excretion and heat production. Significant change in heat dissipation were not demonstrated and the slight fall in body temperature (due to prolonged inactivity), seen in the control experiments was somewhat over-compensated by the drug.

¹ Benedict, F. G., and Tompkins, E. H., Boston Med. & Surg. Journ., 1916, 174, 857.

In each of two additional experiments the subject was allowed to sleep for about an hour. The results did not vary significantly from those recorded above. Since no temperature depression could be elicited in this way it is concluded that sensitivity to antipyretics depends on something different from "central depression."

Acetyl Salicylic Acid and Heat Regulation in Febrile Cases. H. G. BARBOUR. From the Department of Pharmacology, Yale University School of Medicine.

The effects of 0.75 to 1 gram of acetyl salicylic acid were studied upon patients in the New Haven Hospital by the same procedure as that described for normal individuals. The breakfast recommended by Soderstrom, Barr and Dubois¹ was given, the drug being administered three hours thereafter.

Two cases of tuberculosis, one of osteomyelitis, and one of empyema are here reported. All were nearly afebrile at the time of the observations, the average of the initial rectal temperatures being 37.96°C. The following results were obtained:

FOUR FEBRILE SUBJECTS	CALORIES PER SQUARE METER PER HOUR				BODY TEMPERATURE CHANGE
	Produced		Eliminated		
	Control	A-S.A.	Control	A-S.A.	
Average of four experiments	40.2		37.7		°C. +0.18
Average of six experiments		38.8		52.1	-0.81
Change induced by drug		-3.5%		+38.2%	

The average pulse fall was one beat per minute every half hour in the control experiments, but after the drug the decrease amounted to $3\frac{1}{2}$ beats per minute for each half hour. In one case a temporary disturbance in rhythm occurred and in another the rate fell by nearly one-half for a short time.

It is concluded that acetyl salicylic acid does not alter the CO₂ excretion and heat production significantly in febrile cases. In common with most antipyretic drugs, including other salicylates, it exerts its strong antipyretic effect by increasing heat elimination.

Recovery from the antipyretic effect of the drug has been analysed in two further experiments, the observations beginning about five hours after administration. Here also the heat production is but slightly affected, the rapid temperature rise being brought about chiefly by a reduction of heat elimination to about one-half of the normal figure.

Doses of acetyl salicylic acid not antipyretic in healthy individuals appear to exhibit in convalescents with normal temperatures a temperature-reducing potency equal to that seen in the febrile condition. The

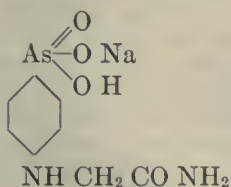
¹ Soderstrom, G. F., Barr, D. P., and DuBois, E. F., 1918, 21, 613.

statement cannot therefore be made that such drugs tend to "stimulate" the heat regulating mechanism.²

On N-Phenylglycinamide-p-arsonic Acid. WALTER A. JACOBS AND MICHAEL HEIDELBERGER. From the Laboratories of the Rockefeller Institute for Medical Research, New York.

For a number of years we have been working in conjunction with Drs. Brown and Pearce on the synthesis of organic arsenic compounds for the treatment of experimental trypanosome and spirochete diseases. Our studies have taken numerous directions all of which have been attempts to depart from the already somewhat exhaustively studied types of aromatic arsenic compounds. Although considerable of our attention has been given to trivalent arsenicals, a great deal of effort was turned to the development of compounds containing arsenic in the pentavalent form as presented by the arsonic acids. If a practicable and efficient drug could be found within this group it would go far toward eliminating certain of the well known practical disadvantages of the usual arseno compounds for as an arsonic acid it should form soluble and stable salts and perhaps offer fewer of the preparative uncertainties than have been the experience with trivalent arsenicals.

In the course of these studies a number of substances have been obtained which have given interesting results. However those obtained with one in particular, the amide of phenylglycine arsonic acid



were such as to demand special attention. Owing to the simplicity of this compound the ease of preparing it, its relatively inexpensive character, stability and solubility and its favorable biological behaviour, a preliminary publication seems warranted.

We wish therefore to preface the biological data to be presented by Drs. Pearce and Brown by a chemical report.

This substance may be prepared by two methods.

1. By boiling a solution of sodium arsanilate with chloroacetamide for one hour, the mixture yielding on cooling phenylglycinamide-p-arsonic acid as colorless crystals. These, after recrystallization are dissolved in dilute sodium hydroxide solution and then precipitated with alcohol as the crystalline sodium salt of constant composition.

2. Phenylglycine arsonic acid (easily obtained by the interaction of the sodium salts of arsanilic and chloroacetic acids) is converted into

² These investigations have been made possible by the friendly coöperation of Dr. F. G. Benedict, and are being financed in part from the Loomis Research Fund of the Yale Medical School.

the methyl ester by boiling in methyl alcohol containing sulfuric acid. The methyl ester is converted into the amide by dissolving the concentrated ammonia and after completed reaction the ammonia is removed. The resulting phenylglycinamide arsonic acid is then precipitated by acidification and after recrystallization is converted into the sodium salt as given under the first method.

The sodium salt is a colorless crystalline substance containing when air dried one-half a molecule of water of crystallization, and 24.57 per cent of arsenic. It dissolves extremely easily in water forming neutral solutions which are perfectly stable. The aqueous solution may be boiled for a reasonable length of time without detectable cleavage of ammonia or arsenic. The large scale preparation of the substance should offer no difficulties and the materials needed are available.

Some Aspects of the Biological Action of N-phenylglycine Amide-p-Arsonic Acid. LOUISE PEARCE AND WADE H. BROWN. From the Laboratories of the Rockefeller Institute for Medical Research, New York.

The monosodium salt of N-phenylglycine amide-p-arsonic acid is easily soluble in water in concentrations as high as 50 per cent and when injected intravenously or into the tissues or body cavities, it produces but slight irritation or local injury and is readily absorbed.

The toxicity of the drug for laboratory animals is comparatively low and on the whole, with the exception of the rat, fairly uniform for the five different species in which it has been used, as may be seen in the appended table. The sharpness of toxic action and the relatively low toxicity for monkeys are two especially noteworthy features of the action of the drug. With lethal doses of A 63, certain signs and symptoms of intoxication develop early, consisting of various nervous phenomena, incoördination of movements, tremors, hypersensitiveness, etc., while metabolic disturbances, loss of appetite, weakness, loss of weight, diarrhoea, etc., develop somewhat later. With doses falling but slightly below a lethal level, relatively slight symptomatic signs of intoxication develop early and disappear rapidly while, in like manner, signs of organic injury are not serious and their repair is prompt.

This feature of the action of the drug has an important bearing upon the tolerance of animals to repeated doses and has been shown in a number of experiments of which the following are typical examples.

Mice. Fifteen mice were injected intraperitoneally at five weekly intervals with comparatively large doses beginning with 2 grams and which were progressively increased to 3 grams. Eleven out of 15 mice survived.

Rabbits. Ten rabbits were injected intravenously at six weekly intervals beginning with 0.75 gram per kilo, increasing to 1.1 grams; 8 out of 10 survived.

Monkey. This animal was injected intravenously with 0.75 gram per kilo and the dose raised on three successive injections to 1.5 grams within thirty days. This animal showed no evidence of intoxication during three months, at the end of which time it was autopsied and no pathological changes attributable to the action of A 63 were found.

The therapeutic action of the drug was studied in experimental infections produced by spirochetes of relapsing fever (*Sp. novyi* and *Sp. obermeieri*) in mice and rats and in experimental syphilis of rabbits. With the first infection, relatively large single doses are necessary to produce any decided effect upon the infection but that there is a definite influence is indicated by positive reinoculation results at the end of sixty days—a standard which is generally regarded as evidence of a valid drug cure. The action of the drug upon experimental syphilis in the rabbit is somewhat comparable. Relatively large doses are required to free the chancre lesions of spirochetes. On the other hand small doses will cause extremely rapid regression and healing of the lesions without, however, causing the total disappearance of spirochetes. In the cases in which the lesions have been completely healed irrespective of whether the spirochetes disappeared or not, we have seen no recurrence of the chancre in the original site.

The therapeutic action of N-phenylglycine amide-p-arsonic acid in the treatment of experimental trypanosomiasis is particularly striking as shown both by its speed of action in the blood infections of mice and rats and by its potency of action in the tissue infection of guinea pigs and rabbits. It has been used in infections produced by *Tr. brucei*, *gambiense*, *equiperdum*, *equinum* and *evansi*, in mice, rats, guinea pigs and rabbits. The majority of experiments, however, have been done with a very virulent strain of *Tr. brucei* which is uniformly fatal in untreated animals and all experiments in the treatment of rabbit trypanosomiasis were done with animals which showed marked signs of the disease. The single curative dose of the drug with this strain of trypanosomes varies from, 0.15 to 0.35 gram per kilo of body weight depending both upon the severity of the infection and upon the animal species employed, as may be seen by the accompanying table.

Relation of single curative doses to the minimum lethal dose of N (Phenyl glycinamide p-arsonic Acid, in the treatment of tr. brucei infections

ANIMALS	MINIMUM LETHAL DOSE PER KILO	CURATIVE DOSE PER KILO	FRACTION OF MINIMUM LETHAL DOSE
	<i>grams</i>	<i>grams</i>	
Mice.....	i. p. 2.0-2.25 s. c. 2.5-2.75 i. v. 2.0	0.275 0.2 0.2	$\frac{1}{8}$ $\frac{1}{12}$ $\frac{1}{10}$
Rats.....	i. p. 0.75-1.75 s. c. 1.0	0.25	$\frac{1}{3}$
Guinea-pigs.....	i. p. 1.5 s. c. 1.5	0.15	$\frac{1}{10}$
Rabbits.....	s. c. 1.1 i. m. 1.1 i. v. 0.75-0.9	0.2* 0.2 0.25-0.35	$\frac{1}{6}$ $\frac{1}{6}$ $\frac{1}{3}-\frac{1}{2}$
Monkeys.....	i. v. 1.0-1.25?		

* Only 2 rabbits used—1 cured, 1 relapsed.

Equally successful therapeutic results in the treatment of rabbits infected with this strain of nagana have been obtained by the use of small repeated doses and animals with extremely severe infections, showing advanced signs of the disease, have been cured by the intravenous administration of 3 doses of 0.2 gram per kilo, on successive days.

Pharmacological Action of Gossypol. C. L. ALSBERG AND E. W. SCHWARTZE. From Bureau of Chemistry, United States Department of Agriculture, Washington, D. C.

Gossypol "acetate" was isolated from the ether extract of gasoline extracted cottonseed kernels, by the method of Carruth.

Though tasteless and insoluble, this substance produces irritation of the pleura peritonium, gastro-intestinal tract, and oedema when injected in oily or in alkaline solution.

A fall in blood pressure, and sometimes a slowing of the heart is produced on intravenous injection of the sodium salt of gossypol. Cats and rabbits die of circulatory failure; respiration is usually unaffected. The most prominent phenomenon observed was the cardiac irregularity, presumably, premature and delayed systoles, skipped beats, without any immediate reference to the injection, but more frequent toward the last. Heart block (2 to 1) as observed once. Four milligrams are usually fatal to rats when injected intraperitoneally in oily solution.

Deaths from this substance may be divided into the three usual classes (1) Acute, in which the circulatory failure is the direct cause. (2) Subacute, in which pulmonary oedema ensues. This was observed in cats after intravenous injection of about 75 mgm. per kilo, death occurring from the second to fourth day. In rats dying about the second day pulmonary oedema can be noted ante-mortem by a red coryza of the nose, and post-mortem by similar material in the trachea. (3) Chronic, observed in rats, and evidenced by cachexia and inanition, occurred after administration by stomach tube of gossypol in oily solution. Oil may be found in the stomach of the rat (post-mortem) which had been placed there a week previous, and gossypol re-isolated therefrom.

Pharmacological Action of Cadmium. C. L. ALSBERG AND E. W. SCHWARTZE. From the Bureau of Chemistry, United States Department of Agriculture, Washington D. C.

Acute toxicological and feeding experiments were performed. Cadmium was given as the chloride, but calculated in terms of the metal.

Our results confirm the conclusions of W. Marme (*Rationale Medicin.*, 1867, xxix, 3rd series, p. 125), concerning which he gave no detail. Cadmium is a powerful emetic, 25 mgm. of the metal usually producing emesis when fed to cats in 100 grams of meat, and 15 mgm. when given in 100 cc. of filtered fish juice. Nephritis is produced by parental administration. For rats, rabbits, and cats the intravenous lethal dose was low (2 to 3.5 mgm. of the metal administered as the chloride). Congulation of protein and necrosis occur at the site of subcutaneous

injection. The most evident pathological change, aside from the orchitis, was the blue testicle of the rat, seen both ante- and post-mortem.

In cats the sinus was found beating after respiration had ceased. The lowest dose of cadmium consistently tolerated by the animals (cats) in our series was 20 mgm. daily. No cumulative effects were noted with lower doses. Vomiting and loss of appetite affected the higher-dosed animals.

With dilutions of 250 per million in the diet of growing rats the gain in weight was not accomplished. The appetite was also affected, but apparently not as much as the growth curve.

Penetration of Mustard Gas into Protoplasm and its Mode of Action. R. LILLIE, R. CHAMBERS AND G. H. A. CLOWES. From the Marine Biological Laboratory, Woods Hole, Massachusetts.

Mustard gas undergoes hydrolysis in aqueous solution with the production of hydrochloric acid and di-hydroxy-ethyl sulphide. The toxicity of an aqueous mustard solution to developing starfish eggs was found to be directly proportional to the amount of mustard still remaining undecomposed in the solution.

Mustard causes necrosis of the cell, the effect being exerted after a latent period of several hours. The conclusion has been reached that mustard penetrated the cell in virtue of its organo or lypoid solubility, and that within the cell mustard absorbed on or combined with essential protoplasmic constituents undergoes hydrolysis with the production of nascent hydrochloric acid, which exerts within the cell a toxic effect far in excess of that which would be obtained with a corresponding amount of hydrochloric acid applied outside the cell.

This conclusion was supported by making injections of fresh undecomposed mustard solutions and solutions of mustard which had undergone complete decomposition, directly into the cell cytoplasm by means of a capillary pipette. The undecomposed mustard solution exerted no more immediate effect than distilled water, but after a latent period caused destruction of the cell just as do fresh mustard solutions applied externally.

The decomposed mustard, however, when injected into the cell caused immediate necrosis, the result obtained corresponding with that obtained with the same concentration of hydrochloric acid and being far in excess of that obtained when decomposed mustard solution or hydrochloric acid is applied externally.

The conclusion was reached that while strong mineral acids failed to penetrate the cell but exert their destructive effect on the exterior, organo soluble substances like mustard and other war gases, served as a means of carrying strong mineral acids into the interior of the cell where they are subsequently liberated with the production of a maximum toxic effect.

The Treatment of Experimental Tetanus in Dogs by Intravenous Injection of Tetanus Antitoxin and of Magnesium Sulfate. A Demonstration. J. AUER AND S. J. MELTZER. From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.

Tetanus was produced by an injection of toxin under the skin of the forehead in dogs. The dose employed produced a local tetanus in less than thirty hours and the animals died invariably in strong tetanus in less than eight days. The early manifestations of a local tetanus consisted in wrinkling of the skin of the forehead and a tonic contraction of the ear muscles. The experimental results were demonstrated by lantern slides thrown on a screen. Several groups of slides were shown.

The first group consisted of four slides. The first slide presented a control dog six days after the injection of the toxin: showing strong general tetanus, trismus and episthotonus. The second slide shows the same dog after an intravenous injection of a moderate dose of a 25 per cent solution of magnesium sulphate: trismus and episthotonus were gone and the general tetanus was considerably reduced. The third slide shows the dog after receiving an additional dose of magnesium sulphate: the general tetanus was entirely gone. The dog was now taken from the board and put on the floor. The fourth slide shows the position of the dog on the floor. It is lying relaxed in a prone position, the head being erect in a normal way. Fifteen minutes later this dog was running about on the floor of the laboratory. These photographs show the strikingly relaxing effect of an intravenous injection of magnesium sulphate upon very strong tetanus. From these experiments it is evident that an intravenous injection of magnesium sulphate is capable of saving life in a short time when the individual may be in immediate danger from asphyxia brought about by a strong tetanic condition of the respiratory muscles. These experiments also demonstrate that the relaxation brought about by magnesium sulphate is not due to a curare-like action of that salt.

The second group consisted of three slides. The first slide shows the control in a state of general but moderate tetanus with a strong trismus. The second slide shows the animal in a dying condition from paralysis of respiration brought about intentionally by an overdose of magnesium sulphate. The third slide shows the same dog a few minutes later standing on its feet in a normal fashion. This striking reversion was brought about by an intravenous injection of 10 cc. of a 2.6 per cent solution of CaCl_2 .

The third group consisted of two slides. The first slide shows two control dogs on the fifth day after the subcutaneous injection of the toxin: one dog is dead and the second is in a very strong tetanus. The second slide shows two experimental dogs on the same day, that is, also on the fifth day after the injection of the toxin, but from the third day on these dogs received twice a day subcutaneously 10 cc. of a 25 per cent solution of magnesium sulphate. The dogs are alive and not in tetanus; they are lying in a prone position greatly relaxed, the heads

erect and nearly in a normal fashion. These experiments show the favorable action of subcutaneous injections.

The fourth group consisted of two slides. The first slide shows two dogs both of which received a lethal dose of toxin five days before. The control dog is lying on the floor in strong tetanus. The second dog received an intravenous injection of antitoxin sixty hours after the injection of the toxin. This animal sits upright in a nearly normal condition. The second slide shows two dogs both of which received intravenous injections of antitoxin sixty-seven and a half hours after the injection of the toxin. One dog is dead; it received antitoxin alone. The second dog stands upright in a fairly normal fashion. This dog received in addition to the intravenous injection of antitoxin, injections of magnesium sulphate.

Action of Local Anesthetics on Striated Muscle. DAVID I. MACHT AND S. KUBOTA (by invitation). From the Pharmacological Laboratory, Johns Hopkins University.

This paper appears in the *Journal of Pharmacology and Experimental Therapeutics* for April, 1919.

Parazol as a Skin Irritant. A. E. LIVINGSTON. From the Hygienic Laboratory, Washington, D. C.

Read by title only.

Pharmacological Action of Mercury Fulminate. A. E. LIVINGSTON. From the Hygienic Laboratory, Washington, D. C.

Read by title only.

Citric Acid as a Constituent of Normal Human Urine. S. AMBERG AND M. E. MAVER.

Amberg and McClure have shown that normal human urine when properly treated yields pentabromacetone and gives a positive Denigès reaction using the method of Salant and Wise. From this they concluded that the urine contains citric acid. Efforts at isolation of this substance from urine were made. A small amount of a substance was obtained which behaved like the dicalciumcitrate described by Henkel.

The Secretion of Urine and Action of Diuretics as Influenced by Blood Flow and Reduction of Renal Substance. E. K. MARSHAL AND A. C. KOLLS. From the Pharmacological Laboratory, Johns Hopkins Medical School.

The results of experiments reported here are a continuation of the problem reported at a former meeting (*Jour. Pharm. and Exp. Ther.*, ix, 346, 1917). The differences in the secretion of urine after section of the splanchnic nerve on one side as compared to the secretion of the other side can be attributed to the increased blood flow through the kidney known to occur after section of the nerve. Section of the splanchnic nerve on one side was used, therefore, to increase the blood

flow through the kidney. Careful compression on the renal artery was used to decrease the blood flow. To reduce the amount of renal tissue, the posterior branch of one renal artery was ligated. When the amount of renal tissue in one kidney is reduced by this method and its secretion compared with that of the normal kidney, about one-half as much water, chlorides, urea, creatinine, and phenolsulphonephthalein are eliminated. However, here the blood flow as well as amount of renal tissue is reduced. By sectioning the splanchnic as well as ligating the posterior branch of the renal artery of one kidney, we have a condition where the amount of renal substance is reduced without much if any reduction of blood flow as compared to the normal kidney of the other side. Using these methods of changing blood flow and amount of renal substance, we find that the substances secreted by the kidney can be arranged in two groups: (1) Water and chlorides which are dependent on blood flow more than amount of renal substance, and (2) Creatinine and phenolsulphonephthalein which are dependent on the amount of renal substance and to a very slight, if any extent, on blood flow for the amount secreted. Urea occupies an intermediate position between these groups, while carbonates resemble chlorides, and sulphates and phosphates, creatinine more than the other group.

Diuretics can be divided into two groups according to the effects produced when the blood flow of one kidney is increased or decreased. When the splanchnic nerve is sectioned on one side, and sodium chloride solution is injected, the difference between the secretion of the two kidneys is magnified. On the other hand when sodium sulphate is injected under the same conditions, the difference in the amounts of urine secreted by the two kidneys is diminished and the two kidneys eliminate nearly the same quantities of urine. Ringer's solution acts similarly to sodium chloride, while sodium nitrate and glucose resemble sulphate in their action.

A Pharmacological Study of Benzaldehyde. DAVID I. MACHT. From the Pharmacological Laboratory, Johns Hopkins University.

In several publications appearing elsewhere the author has described his investigations concerning the pharmacological properties of some benzyl esters on the one hand, and of benzyl alcohol on the other.

Following these studies it was but logical to inquire into the properties of benzaldehyde, a chemical substance closely related to the above. Accordingly, experiments were instituted with the object of determining whether benzaldehyde exhibits the antispasmodic properties of benzyl benzoate on the one hand, and the local anesthetic properties of phenmethylol or benzyl alcohol on the other.

Benzaldehyde is sufficiently soluble in water (0.2 per cent) to admit of experimentation on isolated tissues in vitro. Experiments with solutions of benzaldehyde on various isolated smooth-muscle organs were found to show that benzaldehyde relaxes the tonus and inhibits the contractions of such organs. Experiments with the drug on whole animals and observations of various organs in situ revealed also a sedative effect.

More interesting than the effect on smooth-muscle is the local anesthetic action of benzaldehyde. Experiments with aqueous solutions and more concentrated suspensions or emulsions of benzaldehyde showed that that substance possesses definite and marked local anesthetic properties. Thus it was found that it anesthetizes the sensory nerve endings of the frog's skin, of the cornea, and of the human mucous membranes. Furthermore, benzaldehyde solutions were found to paralyze also nerve conduction.

The toxicology of benzaldehyde has been worked out long ago, owing to its presence, in combination with hydrocyanic acid, in bitter almonds and other plants. As is well known, benzaldehyde is very little toxic, and can be taken by mouth in large quantities without any injurious effects. For this reason, it is official in the United States Pharmacopoeia. The interesting local anesthetic properties of benzaldehyde found by the present author throw light upon the pharmacological action of compound tincture of benzoin and some other drugs. Practically, benzaldehyde is not as adaptable to clinical use as benzyl alcohol, because solutions of it are rapidly oxidized to benzoic acid.

Further Experiences, Experimental and Clinical, with Benzyl Benzoate and Benzyl Alcohol. D. I. MACHT. From the Pharmacological Laboratory, Johns Hopkins University.

The author reports experimental and clinical data collected during the period of about one and a half years, since the first announcement of his studies on benzyl benzoate and benzyl alcohol.

Experimental data. Benzyl benzoate was found to be a depressant also of skeletal muscle, but to a much lesser degree than in the case of smooth muscle.

Experimental evidence and especially clinical observations show that benzyl benzoate is an expectorant. It was also found both experimentally and clinically that benzyl benzoate is a mild sedative for the respiratory center as well as a dilatator of the bronchioles. It is eliminated or excreted not only through the urine but also through the saliva, bronchial secretions, bile, and pancreatic juice. It was especially interesting to find that benzyl benzoate is also slightly anesthetic locally as shown by experiments on sensitive nerve endings and nerve conduction. It has been used as such by one physician in the form of a spray for dressing a tuberculous knee joint.

Benzyl alcohol in addition to being a local anesthetic also exhibits the anti-spasmodic properties of benzyl benzoate on smooth muscle. Being more soluble in water than the benzoate, it can be conveniently used to demonstrate the sedative action of the benzyl group on isolated tissues. The toxicity of benzyl alcohol or phenmethylol is very low and perhaps even less than originally stated. Both benzyl alcohol and benzyl benzoate, especially the former, possess antiseptic properties, and both of them also exhibit a toxic action on worms in vitro (anthelmintic action).

Clinical data. The clinical applications of benzyl benzoate as first predicted by the author, have been corroborated in practice above all

expectations. The author has collected a long series of gastro-intestinal, gynecological, and angio-spastic cases, in which it has been administered with success. Benzyl benzoate has been found very useful in dysentery, spastic constipation, enteralgia, mucous colitis, and other spastic conditions of the intestines and the stomach. It has been found to give relief in spasmodic dysmenorrhoea where all other drugs, and even operative procedures have failed. It may be used to inhibit the premature labor pains of threatened abortion. It has been used successfully in renal colic and biliary colic. Perhaps the longest series of cases are those in which it has been administered for the purpose of reducing high blood pressure. In these cases the effect is longer lasting than that of the nitrites, and the drug may be effective even in cases in which the nitrites failed to produce any lowering of the blood pressure. The author has been informed by a physician that benzyl benzoate is useful in the relief of persistent hiccoughs.

Benzyl alcohol has been used as a local anesthetic successfully in one case of herniectomy, in one case of laparotomy, in one case of castration for carcinoma of the testis, in several operations for hemorrhoids, and in numerous minor surgical operations of every description. Its chief field, however, is in infiltration anesthesia. For surface anesthesia, the aqueous solutions of benzyl alcohol are not very efficient, because of their poor penetrating power into the tissues. In such cases pure benzyl alcohol should be employed. The author is investigating the penetrative powers of benzyl alcohol when applied to mucous membranes both alone and in various solutions. A considerable number of cases have demonstrated its efficiency as a local anesthetic in genito-urinary work. The local anesthetic and antiseptic properties of phenmethylool suggested its trial in dermatology, and it is being tried out under the supervision of the author along those lines. Ointments and lotions of benzyl alcohol have already been successfully used in the treatment of mustard-gas burns and other burns produced by either chemical or physical agents. Inasmuch as benzyl alcohol is more soluble in water than benzyl benzoate, and inasmuch as such solutions exhibit the anti-spasmodic properties of benzyl benzoate on smooth muscle, it has been suggested that solutions of phenmethylool might be administered intravenously to horses for the relief of colic.

The chief point to be borne in mind in connection with benzyl alcohol solutions is that a pure product should be employed. Impure specimens of benzyl alcohol contain more or less benzaldehyde. The latter drug while also anesthetic rapidly oxidizes to benzoic acid and is, therefore, liable to produce unnecessary irritation. Pure benzyl alcohol oxidizes very slowly and can, therefore, be kept for a longer time. The author is conducting a series of observations on the keeping qualities of solutions of benzyl alcohol in various kinds of glass containers, such as ampules of non-sol glass, flint glass, amber glass, etc.

Toxicologically, no cases have been noted or reported. The author has found only two cases which are of interest as demonstrating the low toxicity of both benzyl benzoate and benzyl alcohol. In one case an infant accidentally drank half an ounce of a 20 per cent solution of

benzyl benzoate. This was followed by a swelling of the abdomen, produced by obstinate constipation and distention of the intestines by gas and was promptly relieved by the use of a rectal tube and laxatives. In the other case, in connection with a circumcision operation, through a mistake of an orderly, about 4 cc. of pure benzyl alcohol was injected instead of a 1 per cent solution of the same. This of course produced complete anesthesia, but was followed by a local necrosis of the tissues, such as would follow the injection of pure ethyl alcohol. No constitutional symptoms were noted and the patient recovered completely. One need only imagine what the same amount of a strong solution of cocain would have done in order to appreciate the low toxicity of phenmethylool.

On the Presence of Histamine (β -Iminazolyl-Ethylamine) in the Hypophysis Cerebri and Other Tissues of the Body and its Occurrence Among the Hydrolytic Decomposition Products of Proteins. JOHN J. ABEL AND SEIKO KUBOTA. From the Pharmacological Laboratory, Johns Hopkins University.

This paper will appear in the June issue of the Journal of Pharmacology and Experimental Therapeutics.

The Effect of Epinephrin and Pituitrin upon the Bloodvessels when Injected Subcutaneously in the Ear of a Rabbit. JOHN AUER AND S. J. MELTZER. From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.

It is well known that vascular effect of epinephrin when injected intravenously is of very short duration. Some time ago we reported that injection of epinephrin into the loose subcutaneous tissue at the root of the rabbit's ear produces a striking constriction of all the vessels of the ear which may last in full strength for many hours.

Recently we studied the vascular effect of pituitrin when injected in the same place, that is, in the loose subcutaneous tissue at the root of the rabbit's ear. The effect was markedly different from that of epinephrin; pituitrin does not cause either an outspoken or long-lasting blanching, although the ear vessels of the treated side do not dilate as well during periods of quiet as those of the normal ear. Both ears flush during rest, but the ear vessels of the pituitrin side are always less wide and the dilatation takes place more slowly than in the untreated ear. This difference may last two hours, but after that the pituitrin ear vessels often flush more strongly than the vessels of the control ear, although the final state of vascular dilatation reached is generally the same on both sides.

On the Relation between Degree and Duration of Protein Sensitization in Guinea-Pigs and Dogs. JOHN AUER. From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research, New York.

Guinea-pigs, sensitized by the subcutaneous injection of 0.5 cc. horse-serum and tested after 160 weeks (more than three years), show

a striking loss of sensitiveness to intravenous injection of horse-serum. The animals in their old age, for three years is about their length of life, respond with mild respiratory symptoms only when 0.5 cc. of horse serum is injected into the jugular vein, and recover apparently completely within one to two hours. This reinjection-dose is at least ten times greater than the dose which almost invariably kills when the test is made 9 to 3 weeks after subcutaneous sensitization.

This observation, together with facts previously established, permits roughly the construction of a curve illustrating the effect of time on the degree of sensitization in the guinea-pig, (the animals being sensitized by the subcutaneous injection of 0.5 or 1 cc. horse-serum, tested by intravenous injection, and acute death with typical respiratory symptoms serving as criterion). Sensitization gradually increases after the first injection, so that after 16 days 0.6 cc. serum usually kills in a few minutes, while 0.4 cc. causes only mild non fatal symptoms; on or before 68 days the fatal dose is 0.05 cc., and the same dose is effective 225 days (32 weeks) after sensitization; after 1121 days (160 weeks) the degree of sensitization sinks to a low level and 0.5 cc. horse serum causes only slight respiratory symptoms and not death.

In dogs, sensitized by the single subcutaneous injection of 5 cc. horse-serum and reinjected at the final test with 10 or 20 cc. horse-serum intravenously, a profound anaphylactic fall of blood pressure may be obtained 13 months after sensitization. After the lapse of 35 or 36 months, however, only one dog out of four showed a fall of blood pressure on reinjection, and in this case the fall was moderate (26 mm.) and lasted only two to three minutes. The anaphylactic nature of this drop was established by the fact that no depression of blood pressure occurred when the animal was again injected with horse-serum (antianaphylaxis).

In none of the five dogs tested did the blood show the marked incoagulability lasting for hours which characterizes anaphylaxis in the dog. Even in the dog tested after 13 months, when a perfectly typical, severe fall of blood pressure occurred, the arterial blood coagulated in seven minutes when tested 37 minutes after the test injection of horse-serum, a time when the normal blood pressure level had not yet been re-attained. The normal coagulation time was 3 minutes.

In the other four dogs (sensitized for three years) the arterial blood tested after the reinjection coagulated even more promptly than the control samples.

With dogs, therefore, a powerful typical anaphylactic fall of blood pressure may be obtained one year after sensitization, but not after 3 years; anaphylactic incoagulability of the blood, on the other hand, may be lost within one year and in an animal which still responds typically as far as blood pressure is concerned.

Trinitro Toluene Poisoning. CARL VOEGTLIN, CHARLES H. HOOPER AND J. M. JOHNSON. From the Hygienic Laboratory, Washington, D. C.

An abstract of this work will appear in the Public Health Report in the near future.

Benzylalcohol as a Spinal Anaesthetic. CARL VOEGTLIN AND A. E. LIVINGSTON. From the Hygienic Laboratory, Washington, D. C.

Two to 4 per cent solutions of benzylalcohol in saline injected into the subdural space of dogs produce anaesthesia of the posterior part of the body. The action of benzylalcohol is very similar to that of the cocaine series. When the drug is prevented from reaching the thoracic chord, the blood pressure and respiration are not affected to any marked degree. Injections into the thoracic region are followed by a severe fall in blood pressure and a decrease in the respiratory movements. Application of benzyl alcohol solutions to the fourth ventricle produce a severe fall in blood pressure and a depression of respiration. Respiration may stop very suddenly. Artificial respiration, instituted as soon as natural respiration stops, may save the life of the animal.

Benzylalcohol dissolves in any proportion of olive oil. These solutions also produce spinal anaesthesia and possess the advantage of considerably reducing the diffusion of the drug to the upper chord.

Saline or oil solutions of benzylalcohol produce spinal anaesthesia in normal monkeys.

Action of Certain Drugs on the Output of Epinephrin from the Adrenals.

G. N. STEWART AND J. M. ROGOFF. From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University.

1. In discussing essential points in the technique it was pointed out that in general it is no more possible to demonstrate (or measure) alterations in the rate of epinephrin output by observations which only take account of changes in the concentration of epinephrin in the blood coming from the adrenals, while ignoring concomitant changes in the rate of the blood flow, than it would be to demonstrate (or measure) alterations in the rate of carbon dioxide production in an organ by observations which only took account of the number of volumes of carbon dioxide in 100 cc. of blood but paid no attention to the number of cc. of blood passing through the organ in a given time.

2. We have already reported (*Am. J. Physiol.* 1919, *xlvi*, 22) that strychnine, in convulsant doses, causes a marked and long-lasting increase in the output of epinephrin. It can be added that increases up to ten times the original output have been observed, and that doses well within the therapeutic range and which cause little or no exaggeration of reflex excitability are capable of producing a considerable augmentation in the rate of output. This is true when the drug is administered subcutaneously as well as intravenously. Indications were obtained, in some experiments, that the stage of prolonged augmentation of the rate of output, which constitutes the principal action of the drug, may be preceded by a transient diminution. This phenomenon was best seen with the smaller doses and with subcutaneous administration of the drug, presumably because with the larger doses and with intravenous injection the augmentation of the output comes on so rapidly as to mask any preliminary decrease. The augmentation is associated with a more or less marked increase in the epinephrin concentration even when at the same time the rate of blood flow through the adrenals has been increased, a phenomenon not seen in the absence of the drug.

3. The predominant and by far the most durable action of nicotine whether administered intravenously or subcutaneously, upon the epinephrin output is a depressant or paralysing action. The maximum diminution of the epinephrin output is rather rapidly reached and then there is a more gradual recovery, which when the dose is not too large, proceeds till the original output is approximately attained. At the time of maximum depression no epinephrin at all may be detected in the adrenal vein blood by the test objects chiefly employed (rabbit intestine and uterus segments). The depressant action is preceded by a transient stage of excitation, lasting, as a rule in these experiments, not longer than from half a minute or less to a minute. In this stage the rate of epinephrin output is markedly increased (from 2 or 3 to 10 or 15 times the original output or even more, under our experimental conditions). The latent period of the transient excitation, with intravenous injection of the drug, is very short. In some of the experiments there was evidence that it could not have exceeded a few seconds.

The changes in the rate of epinephrin output are roughly parallel to the changes in the blood pressure caused by nicotine, indicating that when the sympathetic ganglion cells on the efferent vasomotor path are being stimulated or depressed, a corresponding stimulation or depression is being exerted on the efferent adrenal secretory path. The nicotine action develops more suddenly than the strychnine action, as might be expected from the fact that the point of attack of nicotine is the efferent path, of strychnine the central mechanism.

The transient augmentation of the epinephrin output by nicotine may be associated with an increase in the concentration of epinephrin in the adrenal vein blood much beyond the maximum seen with the slowest blood flows in animals simply anaesthetised (with ether, morphine or urethane). The strychnine augmentation of the output has not been observed to be associated with any increase in the normal maximum concentration (something like 1:500,000 in the serum of adrenal blood assayed with rabbit segments).

Confirmatory evidence of the conclusions deduced from assays of the adrenal blood on rabbit intestine and uterus segments has been obtained by a method of auto-assay (collection of adrenal blood for a given time in a cava pocket and study of the blood pressure reactions elicited when the blood is released from the pocket into the circulation), and by other methods.

4. We have been unable to demonstrate any decided and constant effect of strophanthin upon the epinephrin output.

Incidentally it was seen that the epinephrin passing into the blood stream from the adrenals at the ordinary rate can exert a definite action upon the heart. This was clearly shown by the marked effect produced upon the cardiac irregularity evoked by strophanthin when the adrenal blood was temporarily excluded from the circulation or allowed to enter it. Similar observations were made in the case of cardiac irregularity occurring in the absence of strophanthin. It was proved by artificial administration of adrenalin that the constituent in the adrenal blood responsible for the observed effects was epinephrin.

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